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CORRECTIONS

J.R.R.I.M., Vol. 13, Communication 276

Page 101, para. 3, line 2:	- *For*	microspic *read* microscopic
para. 4, line 2:	- *For*	by Dr. Bloomfield, and Dr. van Essen described
	read	by Dr. Bloomfield and Dr. van Essen. Dr. Bloomfield described
„ 103, para. 1, line 14:	- *For*	analagous *read* analogous
„ 104, para. 2, line 4:	- *Omit*	the *before* loose
para. 3, line 1:	- *For*	phosphates
	read	phosphatase
„ 105, para. 1, line 5:	- *For*	absorbed *read* adsorbed
„ 110, line 1:	- *For*	Reserches *read* Recherches
„ 114, under author's name:	*Add*	Centrale Proefstations Vereniging, Bogor
„ 115, para. 4, line 14:	- *For*	mobilished *read* mobilised
„ 124, under author's name:	*For*	Rubber-Stichting, Delft
	read	Indonesisch Instituut voor Rubber-Onderzoek
„ 132, para. 2, line 4:	- *For*	solvatation *read* solvation
„ 138, para. 2, line 8:	- *For*	P/M *read* P/Mg
„ 142, line 8 from end:	- *For*	orgin *read* origin
„ 143, reference (10):	- *For*	Jermijn *read* Jermyn
„ 149	- *Add*	footnote: This work is fully reported in *Trans. I.R.I.* Vol. 27, 290 (1951)
„ 166, para. 2, line 3:	- *For*	Lanmuir *read* Langmuir
„ 169, Table:	- *Add*	the word Clone at the head of column 1
	Add	the number 25 *after* P.B. in column 1
„ 186, para. 3, line 4:	- *For*	theorical *read* theoretical
„ 192, last line:	- *For*	re-injected *read* re-injecting
„ 196, para. 1, line 1:	- *For*	mean *read* means
„ 199, line 3 from end:	- *For*	injections *read* injection
„ 211, line 8 from end:	- *For*	contaning *read* containing
„ 212, line 8 from end:	- *For*	x and y *read* $\bar{x}$ and $\bar{y}$
„ 214, line 4 from end:	- *For*	signifianct *read* significant

(Apologies are tendered for the unusually large number of minor errors which have escaped correction in the editing of this publication—principal corrections only are listed above—Ed.).

PROCEEDINGS

of a

LATEX SYMPOSIUM

held at

THE RUBBER RESEARCH INSTITUTE OF MALAYA,

Kuala Lumpur

on

February 19th—21st, 1951.

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REVIEW OF THE SYMPOSIUM

The symposium opened with an introductory review of the purpose and scope of the conference by the Director of the Rubber Research Institute. The free discussion of scientific results between research workers in the industry was to the benefit of the natural rubber industry as a whole. The scope of the symposium was wide, embracing the physiology, chemistry and technology of latex. On some topics, therefore, discussion might have to be limited, but the conference would gain by acquiring a wider view of the latest progress in latex research and technology. After welcoming overseas visitors, the business of the conference was commenced.

Section I—Physiology of Latex

The papers presented in this section were concerned particularly with the composition and structure of *Hevea brasiliensis* latex, and their relation to both rubber biosynthesis and yield. This was emphasized in his introductory paper by Dr. Schweizer, who drew attention to the presence in latex of organic intermediates which may be used by the tree for rubber synthesis. The work of Bonner in which the path of simple compounds containing radio-active carbon was followed in *Parthenium argentatum* supported the view that rubber was formed from an intermediate of carbohydrate metabolism.

Some methods of influencing yield were then discussed. Dr. Chapman presented data showing that increased yields were obtained by periodic treatment of the bark with synthetic hormones in a palm oil base. The increase was greater for low yielding trees than for high yielders, suggesting that the former may be deficient in a natural hormone. It was pointed out, however, that no very close correlation appeared to exist between natural hormone content (as estimated by the Avena test) and yield. Since the application of synthetic hormone resulted in late dripping it was probable that the increased yield was due to more rubber being drawn per tapping operation than to an increased rate of rubber synthesis. M. Tixier described the effect on yield of copper sulphate injection of the tree. The increase in yield depended not only on the plant material but on the location of the

trees, suggesting ecological influences. Whether the increase in yield was greater when the soil was deficient in copper had not been investigated as yet. In addition to increased yield effected by copper sulphate injections, the latex remained in good condition for a longer period in the field while the Mooney viscosity of the dry rubber was lower, and the modulus of vulcanisates higher. Whereas treatment with hormones tended to produce bark necrosis, no such effect had been observed in the copper sulphate treated trees. There was general agreement that artificial treatments with hormones, copper salts etc. would need to be observed over a longer period before their full effects could be assessed.

The influence of tapping on the composition of latex remote from the tapping cut was described in a communication from Mr. Gooding. Extraction of small samples of latex from various parts of the tree showed that physiological dilution of the latex occurred at great distances (*e.g.* 30 feet) from the tapping cut, a fact which had not formerly been realised. M. Cretin gave an account of changes in the oxidation-reduction potential of latex during the first few hours after tapping. A considerable decrease in potential invariably occurred, which could be arrested by various enzyme inhibitors. Further work would be needed to ascertain whether such changes in potential could be correlated with changes in stability or other technical properties of the latex or rubber.

The session concluded with an absorbing account by Dr. Ruinen of comparative microspic observations on the latex of *Hevea brasiliensis* and other laticiferous plants. It was shown that the occurrence of lutoids (suspended bodies of high phosphorus and nitrogen content, readily distinguishable from the normal rubber particles) in Hevea latex was not unique, but could be observed in the laticiferous systems of several other plants.

Section II—The Latex Hydrocarbon

Important new data on the characteristics of rubber hydrocarbon in fresh latex were given in papers by Dr. Bloomfield, and Dr. van Essen described a form of rubber in Hevea latex, called microgel rubber, which possesses a very high molecular weight as shown by osmometric measurements, but shows an abnormally low viscosity in benzene solution. The explanation of this apparent anomaly is that some form of cross-linking occurs

between the hydrocarbon chains within each latex particle. Rubbers obtained from latices containing a high proportion of microgel rubber are very hard. The proportion of microgel rubber in long rested or previously untapped trees was initially high, in most cases, though certain clones, *e.g.* P.B.186, appeared to be deficient in microgel rubber. As tapping proceeds, the proportion of microgel rubber in the latex from a given tree diminishes, reaching a steady level after about 10 tappings. This fact enables important conclusions to be drawn concerning the area of bark affected by tapping. Although there is often a correlation between plasticity of dry rubber and solution viscosity, Dr. Bloomfield concludes that viscosity cannot be used as a reliable guide to plasticity.

A simple and effective method for determining the molecular weight distribution of rubber in fresh latex was described by Dr. van Essen. Latex is completely soluble in a binary solvent/non-solvent mixture such as toluene/pyridine, provided the proportion of non-solvent is not too high. By increasing the proportion of non-solvent to solvent, partial solution of the rubber occurs, the fraction of rubber with the highest molecular weight being the first to come out of solution. Adjustment of the solvent/non-solvent ratio therefore enables the molecular weight of a series of fractions to be determined, from which a molecular weight distribution curve can be constructed. Drs. Bloomfield and van Essen both show that the molecular weight of latex rubber can reach very high values (several millions).

Section III—Colloidal Properties of Latex

The papers in this section were concerned particularly with the relation between latex composition and stability. It had been found previously by Homans and van Gils that unammoniated latex could be separated into two fractions by centrifuging, a white fraction and a denser yellow fraction containing essentially all the lutoid bodies. Remnants of the yellow fraction may be found in the sludge obtained during centrifuging of ammoniated latex. The importance of the yellow fraction in controlling the stability of unammoniated latex was emphasized by Mr. Cook and Dr. McMullen who showed that it was the source of highly surface active material. Under normal non-sterile conditions the yellow fraction coagulates more

rapidly than the white fraction. If the latex is collected and maintained sterile, the yellow fraction still coagulates within a few hours, whereas the white fraction does not undergo spontaneous coagulation. This was made clear in a communication by Dr. McMullen who also described a reliable technique for the collection of latex under sterile conditions which should be of great value in latex research. Coagulation of the yellow fraction in the absence of bacteria is almost certainly an enzyme reaction since the coagulation can be inhibited by enzyme poisons. The mechanism of the enzyme coagulation and the particular enzyme(s) involved have not yet been fully explored, but Dr. McMullen suggested an interesting, though speculative mechanism somewhat analagous to that of blood clotting. In a contribution to the Symposium by Dr. van Gils, a method was proposed for eliminating the influence of the d.r.c. on the viscosity of the latex. In this way other factors determining the viscosity, as for instance the yellow fraction, and the clone, appear more pronounced. An interesting suggestion by M. Compagnon was that a connection may exist between the proportion of yellow fraction in latex and the incidence of brown bast.

The pre-coagulation of latex in wet weather was shown by Mr. Cook and Dr. McMullen to be due to contamination of the latex by chemical substances from the bark.

Dr. Paton discussed the anomalous properties of G.1 latex. Its instability appears to be closely connected with the high ratio of Mg to P in the latex, though the presence of organic acidic compounds in the serum may be a contributory factor. Addition of phosphate was found to be effective in raising the stability which was enhanced by the further addition of fatty acid ammonium soaps.

Work by Mr. Philpott also suggested that latex stability is related to the P:Mg ratio; for maximum stability, a ratio equal to about 1.5 appears to be desirable.

Section IV—Analysis and Non-Rubber Compounds

Some preliminary investigations into the composition of the phosphatides and proteins of fresh latex were described in papers by Mr. Resing, Mr. Drake and Mr. Sekhar and Dr. McMullen. These non-rubber components are important not only as natural stabilisers for

latex but as a possible source of variability in raw rubber and its processing behaviour. Both the cephalin and lecithin fractions of latex phosphatides appear to contain appreciable amounts of combined sugars, including galactose and glucose. Combined serine and inositol were also detected by Mr. Resing (but not combined quebrachitol). Paper partition chromatography was used by Mr. Drake for identifying the constituent amino acids present in latex proteins. An interesting feature was that the latices of three different clones (G.1, R.R.I. 501, and Prang Besar 186) gave identical chromatograms.

Mr. Sekhar and Dr. McMullen gave an account of their work on the fractional separation of latex proteins, most of which appear to be present in latex in the form of lipo-protein complexes in the loose combination with carbohydrate.

The presence of phosphates in latex was confirmed by Mr. Cheah Sein Liat. There is no evidence as yet to show whether latex phosphatase affects in any way rubber formation or latex stability; it does not appear to play any part, however, in the breakdown of phospholipids.

Much discussion ensued following an account by Mr. Drake of the methods now in use at the R.R.I. for determining the inorganic constituents of latex. The importance of the ashing procedure was emphasized. Gentle ignition in an alkaline medium followed by digestion of the ash in acid solution is essential for the correct determination of phosphorus. Similarly, very slow ignition of an acidified rubber sample is required for the estimation of metallic constituents.

Section V—Latex Technology (1)

The last day of the symposium was devoted to technological latex problems. In a paper by Mr. van den Tempel it was shown that the size-frequency distribution of ammoniated latex particles contain a maximum far below the viscosity limit and can be expressed by an equation from which the average volume or average surface area of the particles may be calculated. Other quantities which may be derived are the number of particles in a given volume of latex or the total surface area of all particles. To do this it is necessary to measure independently the rubber content of the latex and one of the quantities

mentioned above, *e.g.* the total surface area of all particles. A promising method of determining the latter experimentally was described by Dr. Cockbain. In principle it consisted of measuring the amount of a suitable synthetic soap which could be absorbed on the rubber particles. Account had to be taken of the soaps, proteins, etc., already present in Hevea latex.

A series of papers on the stability of ammoniated latex followed. Mr. Pidford described a method of determining the stability of latex to zinc oxide in which the latex-ZnO mixture was maintained at a given temperature for one minute. The highest temperature at which the mix does not gel under these conditions is a measure of its stability.

An important communication by Drs. Kraay and van den Tempel on the mechanism of gelation of ammoniated latex in presence of ZnO was presented by Dr. de Decker. Evidence was given indicating that the zinc-ammonium complex salts which are slowly formed do not themselves destabilise latex. However, zinc ions are produced by decomposition of the complex salts (particularly at elevated temperatures) and the resulting formation of insoluble zinc soaps in the latex then results in gelation or coagulation.

Dr. Cockbain gave an account of work he was carrying out on the gelation of ammoniated latex by means of polyvinyl methyl ether. This polymer is readily soluble in water below about 34°C but is precipitated from solution at higher temperatures. When present in latex-zinc oxide mixes it renders the latex heat-sensitive above 35°C. Data was given showing that displacement of natural proteins by the polymer molecules from the surface of the rubber particles was a necessary preliminary to heat sensitisation. Dr. Cockbain also described work on the creaming of synthetic emulsions designed to throw further light on the mechanism of latex creaming.

Section V—Latex Technology (2)

MM. Compagnon and Cretin reported some interesting results of a preliminary nature indicating correlations between the copper or nitrogen content of different latices and their DRC and also total solids. A relationship between these properties and the modulus of the rubber had also been observed. Further work is proceeding.

Dr. McMullen described experiments in which latex had been preserved for long periods in the absence of ammonia, using non-ionic soaps and an effective enzyme poison. The author concluded that complete coagulation of the latex was normally due to bacterial contamination, but that precoagulation was probably caused by enzymes native to the latex acting preferentially on the yellow fraction. Similar conclusions were reached by Mr. Cook who had also found that sheet rubber prepared from latex temporarily preserved with low concentrations of formaldehyde (0.1%) were practically unaffected in appearance or technical properties.

Messrs. Philpott and Sekar described an accurate method for determining the volatile acids in latex. Results were given showing that the volatile acid content of inadequately preserved latex changed much more rapidly than either the KOH number or the electrical conductivity.

The symposium concluded with a description by Dr. McMullen of some microscopical studies of the structure of thin films of coagulated rubber. In the initial stages of coagulation a high percentage of the rubber particles appeared to be in a discrete form, connected only at points where local displacement of the protective film from the surface of the particles had occurred. The tensile strengths of coagula were found to be almost proportional to the DRC of the latex.

Summary

A review of the symposium as a whole gives a clear picture of the most important trends in present-day latex research and technology.

A new approach to the question of increased yield is being made (involving physical or chemical treatment of the tree) which may prove a valuable adjunct to selection of plant material and agricultural methods. Whether the chemical treatments so far attempted result in an increased rate of rubber formation over long periods, or whether they are to be regarded only as temporary stimuli remains to be proved. It should be remembered, however, that work of this type is only in the early stages of development and by no means gives a final picture of its possibilities.

On the problem of latex stability, it was clear that many members of the conference attached great importance

to a more detailed study of the yellow fraction of latex, which is undoubtedly much more unstable than the white fraction in latices which have been studied so far. The reasons for this relative instability are still not completely understood, though some of the papers presented during the symposium show that intensive efforts are being made to unravel this difficult problem.

The discussions on analytical procedures made it abundantly evident that the closest contact must be maintained between different laboratories to ensure that techniques are standardised as far as possible, otherwise contradictions and confusion may well arise.

The contributions during the final session on latex technology were particularly welcome, since they forged a link between the producer and user of latex. It was encouraging to note that intensive efforts are being made on the technological side to control and, if possible, improve the quality of natural latex.

E. G. Cockbain.

INTRODUCTION TO THE SYMPOSIUM

When the first stages of reorganisation of the work of the Rubber Research Institute of Malaya had been completed after the Japanese occupation and it was possible to devote full attention to planning the course of future experimental work, a general programme of research to cover the period 1948 to 1952 was prepared. This programme was carefully considered by the Board in consultation with other organisations for research on natural rubber in the United Kingdom, namely the London Advisory Committee for Rubber Research (Ceylon and Malaya), and the British Rubber Producers' Research Association.

Eventually a co-ordinated programme was prepared which presented a complete statement of proposals for future expansion of research and development work for natural rubber by the principal organisations for such work financed by the Malayan Rubber Fund. Details of the plan are published elsewhere; for the present it is sufficient to refer to the development of the research and experimental work on natural latex.

It is now fully realised that the position of natural rubber as an essential raw material of commerce has been threatened seriously by the rise of the American synthetic rubber industry, first during the war years and now in its post-war development. Whereas before the war the emphasis in research in the East was upon improvement in the quality of the planting material and of methods of production directed towards reducing production costs, we are now concerned not only with costs of production but also with the quality of the product in comparison with its synthetic competitors. Thus since the war we have tried to increase our research effort in the fields of rubber chemistry and technology, to learn much more about the fundamental properties of rubber and latex, and to explore methods of improving or modifying them in useful directions.

In 1949 the Board of the Rubber Research Institute of Malaya authorised the Director to arrange special meetings from time to time for the purpose of review and discussion of particular subjects of research and experimental work. This arrangements is in continuation

of the policy developed before the war when the Rubber Research Institute organised a first conference of Directors of Rubber Research Organisations in the Far East. In September 1949 the Rubber Research Institute of Malaya arranged a conference of the same kind, but widened its scope by providing for full discussions of an important section of the research field concerning "The Quality and Methods of Grading of Natural Rubber." To that meeting not only were research colleagues invited, but technical men employed in the industry; members of planting associations, and market organisations also attended and contributed much to the discussions. A full account of the meeting is presented in the *R.R.I. Journal Communication* Vol 12 No. 269.

The present meeting has been arranged to provide more particularly for discussion of current research work on latex; we hope to include both botanical and chemical studies. In this field, apart from the notable application of the results of scientific work on methods of concentration and preservation of latex, there is an ever increasing field of purely investigational work which may appear to have little immediate application to practice. For this reason this Latex Symposium may deal largely with matters of lesser appeal to the producers than those discussed at the previous meeting in September 1949. Its principal object is to bring together those who are at present carrying out research in the field of natural rubber latex. Much of this work must be done in the East at the actual seat of formation of latex in the tree, and other problems can be tackled more efficiently using preserved latex in the consuming countries. Between the workers in the entire field there is a need for, and obvious advantage in, close co-operation.

We are fortunate in being able to welcome as members of our symposium representatives of the principal organisations for rubber research in other countries.

Indonesian Rubber Research Institute, Bogor, Indonesia:
Dr. Ir. G. J. van der Bie, Dr. G. E. van Gils, Drs. W. J. van Essen, Mrs. Ir. L. N. S. de Haan-Homans

Centrale Proefstations Vereniging, Bogor, Indonesia:
Dr. J. Schweizer, Drs. G. Verhaar

Treub Laboratory, Botanical Garden, Bogor, Indonesia:
Dr. (Miss) J. Ruinen

The General Experiment Station of the A.V.R.O.S., Sumatra:
Ir. O. B. Schrieke, Ir. C. Vervloet

Institut des Reserches sur le Caoutchouc en Indochine, Laikhé, Indo-China:
M. P. Compagnon, M. P. Tixier, M. C. Cretin

Rubber-Stichting, Delft, Holland:
Dr. H. C. J. de Decker

British Rubber Producers' Research Association, Welwyn Garden City, England:
Dr. E. G. Cockbain

The number of contributions and the wide field covered speaks well for the success of the meeting and indicates very strongly that such meetings serve a most valuable purpose. The opportunity to survey and discuss with others, work in the same field provides a stimulus that cannot but have a beneficial effect on the progress of natural rubber production.

C. E. T. Mann.

PROGRAMME OF THE SYMPOSIUM

Monday, 19th February—Morning Session:

Introduction

SECTION I—*Physiology of Latex*

Paper No.	*Author*	*Title*	*Presented by*
1	J. Schweizer	Latex and Growth	J. Schweizer
2	G. W. Chapman	Plant Hormones and Yield in *Hevea Brasiliensis*	G. W. Chapman
3	E. G. B. Gooding	Preliminary Studies in the Physiology of Latex	C. C. T. Sharp
4	C. Cretin	A Study of the Redox System of *Hevea Brasiliensis* Latex	C. Cretin
5	P. Tixier	The Injection of Trace Elements, especially of Copper in the form of Sulphate, into the *Hevea Brasiliensis* Tree	P. Tixier
6	J. Ruinen	Comparative Microscopical Observations on Latices of Hevea and other plants	J. Ruinen

Monday, 19th February—Afternoon Session:

SECTION II—*The Latex Hydrocarbon*

7	G. F. Bloomfield	Characteristics of Rubber in Latex of Untapped Trees and in Branches of Trees in Regular Tapping	G. W. Drake
8	W. J. van Essen	Molecular Weight Distribution of Rubber in Fresh Hevea Latex of Different Clones	W. J. van Essen
9	G. F. Bloomfield	The Rubber Hydrocarbon in Hevea Latex	G. W. Drake

Tuesday, 20th February—Morning Session:

SECTION III—*Colloidal Properties of Latex*

Paper No.	*Author*	*Title*	*Presented by*
10	A. I. McMullen	The Extraction of Latex under Sterile Conditions and some properties of Sterile Latex	A. I. McMullen
11	G. E. van Gils	The Contribution of the Yellow Fraction to the Viscosity of Latex	G. E. van Gils
12	A. I. McMullen	Some Aspects of Theories of Spontaneous Coagulation	A. I. McMullen
13	A. S. Cook and A. I. McMullen	The Lutoid Fraction and the Stability of Hevea Latex	A. S. Cook
14	F. J. Paton	Latex Stability	F. J. Paton
15	M. W. Philpott	Latex Stability and Composition	G. W. Drake
16	A. S. Cook and A. I. McMullen	The Pre-Coagulation of Hevea Latex in Wet Weather	A. S. Cook

Tuesday, 20th February—Afternoon Session:

SECTION IV—*Analysis and Non-Rubber Compounds*

17	W. L. Resing	Phosphatides of *Hevea Brasiliensis* Occurrence of Sugars	L. N. de Haan-Homans
18	G. W. Drake	The Determination of the Inorganic Constituents of Hevea Latex	G. W. Drake
19	G. W. Drake	The Amino Acid Constituents of the Protein of Hevea Latex	G. W. Drake
20	B. C. Sekhar and A. I. McMullen	A Note on the Fractional Separation of Latex Proteins	B. C. Sekhar
21	Cheah S. L.	A Note on the Phosphatase in Latex	Cheah, S. L.

Wednesday, 21st February—Morning Session:

SECTION V—*Latex Technology (1)*

Paper No.	*Author*	*Title*	*Presented by*
22	M. van den Tempel	The Particle Size of Oil-in-Water Emulsions	H. C. J. de Decker
23	E. G. Cockbain	A Method of Determining the Specific Surface Area of Rubber in Latex	E. G. Cockbain
24	G. M. Kraay and M. van den Tempel	Mechanism of the Gelling of Latex by Zinc Compounds	H. C. J. de Decker
25	J. H. Pidford	A Chemical Stability Test for Latex	J. H. Pidford
26	E. G. Cockbain	Studies on the Creaming of Latex and Synthetic Emulsions	E. G. Cockbain
27	E. G. Cockbain	Heat Sensitisation of Latex and Synthetic Emulsions by Polyvinyl Methyl Ether	E. G. Cockbain

Wednesday, 21st February—Afternoon Session:

SECTION V—*Latex Technology (2)*

28	P. Compagnon and C. Cretin	Correlations of Some Constituents of Latex and Technical Properties of the Rubber	P. Compagnon
29	A. I. McMullen	The Preservation of Latex at Neutral pH	A. I. McMullen
30	A. S. Cook	Studies on Temporary Preservation of Hevea Latex	A. S. Cook
31	M. W. Philpott and K. C. Sekar	The Determination of Volatile Acids in Latex	K. C. Sekar
32	A. I. McMullen	The Structure and Strength of Fresh Coagula	A. I. McMullen

PART 1

ABSTRACTS OF PAPERS

WITH DISCUSSION

Paper No. 1.

Latex and Growth

J. Schweizer

It is an established fact that tapping retards the growth of *Hevea Brasiliensis*. In an earlier publication ("Hevea Latex as a Biological Substance"—*Archief o.d. Rubber Cultuur* Vol. 26 1949, 345) the author drew attention to the competition which exists between growth processes at the meristems and the regeneration of rubber in the latex vessels and stated that rubber regeneration is closely related to growth, both processes taking place at the expense of the daily assimilation current from the leaves.

In growth reactions the carbohydrate breakdown is an exceedingly intricate complex of enzymatic reactions. The intermediates in the latex serum may be used as building blocks in further synthesis of compounds present in it. These syntheses take place in an almost ideal condition in latex; an almost perfect buffer solution, a suitable, balanced redoxsystem, in which already many enzymes are found (oxidising enzymes, phosphatase, phosphorylase, etc.).

The non-rubber constituents of latex probably play a most important part in polymerisation processes leading to the formation of rubber.

The phospholipids/protein-complexes may furnish the matrix for rubber formation (Altman's theory) and may not act as carriers for isoprene from cell to cell; polymerization process may take place according to Harkin's theory. Supply of raw material for rubber formation and emulsion polymerization thereof, are two different processes, as are their locus and mechanism. The various serum substances will furnish the building blocks for the structure necessary for these processes which will take place inside the latex vessels and not in the plasma of these, as assumed up till now. Of course, these substances originate from protoplasma. These ideas agree with Frey Wyssling's supposition of plastid-like rubber formation.

The composition of latex in young latex vessels differs from that in older ones, in accordance with the more active metabolism of the surrounding growing

tissues. This difference is not due to infiltration of "lutoids" or other fluids from phloem-sap, but is inherent to the young latex tubes. The composition of the latex will give a picture of its regeneration capacity. Young or old tissues (organs) of a tree will form or regenerate rubber (primary or secondary rubber formation) according to their latex composition. Further investigations concerning rubber formation must take into account the actual place on the plant from which the latex has been extracted.

Formation of new rubber after tapping is to be looked upon as a regeneration process resembling tissue-growth; all kinds of enzymatic biocatalysed processes may thus occur. Since too little is known about these reactions to obtain some promising insight into one of the many pathways, special attention is to be paid in future to the phosphorus quebrachitol complex in latex, as a probable carrier of energy, the already mentioned phospholipid protein complex being different from this one in function, structure and composition.

Wound regeneration is a hereditary quality and within a species the different organs vary in regeneration capacity; the same holds good for latex rubber regeneration. The process of regeneration is probably linked with another gene than that determining ordinary tissue growth. This may be the reason why the laticiferous capacity of the stock is influenced in all circumstances by the regeneration capacity of the scion, but not by the growth.

Growth and regeneration are biochemical phenomena, based on enzymatic catalytic processes; therefore, they may be influenced by phenocopical or paramorph substances (environmental factors with any corresponding genetic alteration). However, all these factors will be dependent on the carbohydrate reserves, the breakdown products of which will start the forming of new rubber, therefore photosynthesis will play a paramount role in rubber formation. Hevea regenerates an excretion product using and containing the best material, namely the daily current of assimilates. By heavy tapping the growth is retarded on account of insufficient supply of assimilates by the daily current; more deeply situated starch reserves will be mobilished. This is also an individual, specific quality of a tree and is of great importance in view of the fact that the whole tree is active in supplying reserves for rubber formation. If tapping is too heavy, the tissues

are exhausted and consume themselves; water content of latex is excessively increased and brown bast is the result.

The very important results obtained by Bonner etc. with labelled carbon are in accordance with the view that the precursors of the rubber molecule originate from the intermediates of carbohydrate metabolism.

The changes in the composition of latex which occur during "wintering" run parallel to the specific changes in metabolic reactions in the cells of young tissues. These reactions may also influence the rate of polymerisation of the rubber molecule.

Paper No. 2.

Plant Hormones and Yield in *Hevea Brasiliensis**

G. W. Chapman

(Chemara Central Research Station, Malaya)

This work was started in 1937 following the widespread use of cow dung mixtures by Asian planters, both to improve bark and increase yield. The conclusion was reached that the common factor in all these mixtures was plant hormones, but under certain conditions sulphur was able to form a highly beneficial organic compound. It was found that application above the tapping cut generally reduced yield while application below increased it.

After the war by the use of synthetic plant hormones such as naphthalene acetic acid and 2:4-dichlorophenoxy acetic acid much greater effects were obtained. The best results were obtained using palm oil of a definite acidity as a base. These effects for Stimulex—the standard product used on the Guthrie estates—are described for various types of rubber and different yield groups in seedling rubber. The lower yielders give a greater percentage increase than the high yielders amongst seedlings.

A comparison between these effects and the behaviour of trees under tapping suggested the hypothesis that hormones play an important part in yield capacity.

This theory has been developed since 1948, and recently the hormone or hormones have been extracted from latex and estimated by the Avena test. The routine estimation of the hormones present in latex from field experiments is in progress and has shown that the hormone content of latex varies with the clone, and the area and the tapping system. If rubber is rested for a time and then tapped daily the hormone content of the latex is at first very low, but rises to a maximum after 4 to 6 days.

It is suggested that hormone is produced in response to wounding and in this connection it is significant that the application of synthetic hormones also produces late dripping as does fire damage and tapping over a large area as in full spiral.

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* *This work is fully reported in Part II of this publication.*

Paper No. 3.

Preliminary Studies in the Physiology of Latex*

E. G. B. Gooding

(Rubber Research Institute of Malaya)

By the use of a sensitive dendrometer designed by E. E. Pyke it has been found that tapping led to the contraction of the trunk of a rubber tree in the region immediately below the tapping cut, there being a time lag before the contraction appeared at points one foot and two feet below the tapping cut. With increasing distance from the cut, the contraction becomes progressively less.

Ferrand's technique for taking samples of latex typical of that existing in the latex vessels after critical examination, was suitably modified. Using this technique it can be shown that tapping leads to dilution of the latex in the latex vessels below the tapping cut, and to a distance above the cut. The dilution effect is found to spread downwards in the latex system at a speed of 2 inches per minute, and in a lateral direction at a speed of between $\frac{1}{4}$ and $\frac{1}{2}$ inch per minute, within the area of bark studied. It is found that when a tree is brought into tapping for the first time, or after a long period of rest, tapping results in dilution of latex throughout the whole tree.

The implications of these observations are discussed.

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* *This work is fully reported in Part II of this publication.*

Paper No. 4.

A Study of the Redox System of *Hevea Brasiliensis* Latex*

C. Cretin

(Institut des Recherches sur le Caoutchouc en Indochine)

Following on the work of van Dalfsen and de Haan-Homans, the decolouration of methylene blue by Hevea latex has been examined.

It has been observed that latex examined immediately on leaving the tree does not provoke decolouration and the phenomenon takes place only after a certain time of keeping and intensifies.

An attempt has therefore been made to study the oxidation-reduction potential (O.R.P.) of latex and its alteration with time. A few minutes after leaving the tree the latex has a positive O.R.P. in the neighbourhood of + 0.150 vols, and this decreases quite rapidly (the decolouration of methylene blue is then observed) and becomes negative, reaching a stable level generally near —0.100 volts. This change is complete about 10 hours after tapping.

The effect on this change of various enzyme inhibitors has been examined and an inhibiting action has been found especially by heavy metal ions and monoiodoacetate and arsenic ions, these latter being specific for thiol groups.

It is therefore believed that the O.R.P. is dependent on the equilibrium:

$$2\ \text{R.SH} \rightleftharpoons \text{R.S.S.R.} + \text{H}_2$$

The SH groups can belong to such amino-acids as cystein or to such tripeptides as glutathione, which are themselves engaged in more complex combinations.

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* *This work is fully reported in Part II of this publication.*

Paper No. 5.

The Injection of Trace Elements, especially of Copper in the form of Sulphate, into the *Hevea Brasiliensis* Tree*

P. Tixier

(Institut des Recherches sur le Caoutchouc en Indochine)

In a publication appearing recently in Rev. Gen. Caout., Compagnon and Tixier have shown that the injection of a few grams of copper sulphate into Hevea brings about an increase in the production of rubber, which seems to last from three to five months, and which is in the neighbourhood of 40% to 60% over the controls. It is further observed that the rubber content of the latex of the injected trees is less than that of the control latices.

The object of the present communication is to bring forward some new information relative to this increase in production in the light of results from experiments carried out in the course of the year 1950. The stimulation of production is observed on trees of very different ages but an increase of the order of 50% cannot be considered as a general rule. Some very different reactions are observed according to the plant material used and the same clones have not reacted in the same way in different locations, which suggests ecological influences. Some results are given concerning the optimum dose of copper sulphate, the influence of repeated injections on the same trees and the injection of dry trees.

Experiments made on a semi-industrial scale have allowed the latex properties of the injected trees to be studied. Stability in the field appeared better, the modulus of the rubber obtained is significantly increased and the Mooney viscosity is lower.

Summarised indications are given on injection experiments with various other elements.

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* *This work is fully reported in Part II of this publication.*

Paper No. 6.

Comparative Microscopical Observations on Latices of Hevea and other Plants *

J. Ruinen

(Treub Laboratory of the Botanic Garden, Bogor, Java)

By a special microtechnique the lutoids of Hevea latex have been structurally demonstrated in the latex vessels. As in the latex itself they are observed in the form of globules distinctly separate from the rubber particles with a well-developed membrane enveloping a viscous colloidal interior content. The membrane itself contains small rubber particles, and is coloured equally well with rubber stains as with protoplasm stains. The colloidal contents are tintable with rubber dyes.

Outside the latex vessels the lutoids soon disintegrate. The lutoid substance either seeps through the membrane as a liquid, or—after considerable swelling—bursts from it as a naked globule. It sediments slowly. Gelation starts immediately and proceeds gradually. Finally all rubber components are incorporated in the coagulum. Much depends on the reaction of the milieu, but in both alkaline and acid milieu, as well as in alcohol, a part of the lutoid is redissolved. Only in a small range between pH 5.6-7 is the structure more or less stable. This phenomenon suggests a different composition from that of the original globule.

The rather surprising fact that this structure in Hevea latex had been overlooked for so long raised the question of whether or not identical bodies occur in other latices as well. This proved to be the case. All gradations of dispersion were found. Latices of the Hevea type with big aggregates or single lutoids possessing a distinct membrane, besides freely suspended rubber particles, occur but are scarce. Other latices contain only structureless lutoid matter which is actually comparable with the fluid lutoid material and naked globules in decomposing Hevea latex, and (as in this latex) gelates in ridges and banks (Palaquim and Achras). In the fixed plant material, distinct vacuolic structures, from which all the contents have been washed out, are observed in the protoplasmic layer of the latex cells. They present a likeness to the lutoid membrane.

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* *These investigations will be published in Annales bogorienses.*

On the other hand, in other latices the lutoid seems to be missing and the stable latex is a fairly homogeneous suspension of "rubber" globules (Ficus spec.). When treated with rubber solvents or alcohol, however, the original globules lose their contents and shrink. The contents separate into a highly stainable oil-like substance, a slightly tinting oil-like material (probably lutoid substance), small dust-like particles (rubber or resin) and, furthermore, several crystalline organic and inorganic compounds. At this stage gelation sets in. The remaining membrane shrivels like a collapsed balloon. In the anatomical specimen the different components are also observed. The soluble matter is for the greater part washed from the latex cells and only the collapsed membranes are found in the vessels. On the whole the structure seems identical, though more coarse, than the lutoids in Hevea latex, but moreover contains rubber and rubber-like substances.

In conclusion, it seems evident that Hevea latex holds an intermediary position in a range of latices of varied structure, in which Achras and Palaquium form one extreme and Ficus the other. In the first case the lutoid membrane remains adhered to the protoplasmic layer of the cells and is never found in the serum in which all other components, rubber, resins and lutoid substances are present. In the second case all the afore-mentioned components are assembled in one stable body and the lutoid membrane is a coarse structure. Finally, it has been suggested that, considering the labile condition of the lutoids and consequently of the Hevea latex as a whole, another plant may prove to be more suitable for further investigations concerning the site of rubber formation.

Literature

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Paper No. 7.

Characteristics of Rubber in Latex of Untapped Trees and in Branches of Trees in Regular Tapping *

George F. Bloomfield †

(Rubber Research Institute of Malaya)

The discovery that the latex in some untapped or long-rested trees, and in the branches of some trees in regular tapping, contains a high proportion of microgel enables important conclusions to be drawn as to the area of bark affected by tapping since when an untapped tree is brought into production the microgel latex contained in a considerable area of the tree is replaced by normal latex. Certain clonal species, however, notably P.B.186, do not contain very much microgel latex even when old untapped trees are examined. There is a tendency for the tapping of high yielding trees to affect the latex of a greater area of the tree than in low yielders.

Microgel latex has certain novel characteristics which facilitate its identification. Its rubber content can be brought into solution in benzene to give solutions of very low inherent visocity. It can be shown that the rubber is not of low molecular weight, and the osmotic pressure of its solutions is extremely low. Rubbers obtained from microgel latex are very hard.

When a tree containing microgel latex is brought into tapping the inherent viscosity of solutions of its latex increases steadily while the rubber obtained from it becomes progressively softer. After about ten tappings the latex becomes normal.

When two tapping cuts are opened on the same tree the latex drawn from the upper cut gives much harder rubber than that from the lower cut although the inherent viscosities of rubber from the two cuts are not initially different. Differences in the proportion of mineral constituents are found in the latices from the two cuts, the bottom-cut latex containing more phosphorus and potassium but less magnesium than the top-cut latex.

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† *Seconded from the British Rubber Producers' Research Association.*

* *This work is fully reported in* J. Rub. Res. Inst. Malaya, Vol. 13, Communication Nos. 271, 272 and 273.

Paper No. 8.

Molecular Weight Distribution of Rubber in Fresh Hevea Latex of Different Clones

W. J. Van Essen

(Rubber-Stichting, Delft)

It is known that all high polymers are mixtures of macromolecules which differ greatly in size. The molecular weight distribution ratio of such polymers is very important on account of its effect on the physical and chemical properties (plasticity, tensile strength and rate of cure). Several fractionation methods have been developed and in a very extensive review Cragg and Hammerschlag discussed these methods (*Chemical Reviews* 39, 1946, 79). The most widely used fractionation methods are those of fractional precipitation and fractional solution.

Almost all the information in the literature on the mol. weight distribution of rubber deals with solid rubber (crepe, sheet etc.) but it is also important to know the native state of rubber in fresh latex, e.g. before any treatment is applied. Latex is completely soluble in a binary system of a rubber solvent/non-solvent mixture and some figures on the average mol. weight of the whole rubber in fresh latex of different clones have been published by Van Essen (*Recueil* 69, 1950, 753) using a toluene-pyridine mixture (80/20 by volume). The conclusion of this paper was that there is some relation between the plasticity (measured according to Hoekstra D 15′ 70°) and the mol. weight of rubber. Clone Avros 385, for instance, gives a hard rubber with a high mol. weight and the clone Glenshiel 1 gives a soft rubber with a low mol. weight.

Latex-rubbers of the same average mol. weight, however, have not always the same plasticity and, therefore, a fractionating method for rubber in fresh latex in very important. Our object was not to obtain precise distribution curves but primarily to develop a rapid and convenient procedure which could offer information on the relative amounts of low, intermediate and high mol. weight fractions of different clonal latices.

The fractional solution or fractional precipitation methods are very time-consuming and care should be taken that no changes in the polymer molecule are pro-

duced by outside agents (oxygen, light). Sebrell (*Ind. Eng. Chem.* 35, 1943, 736) investigated butadiene-styrene polymers using both the customary "successive" extraction and a "simultaneous" extraction method. He observed good agreement between the results of these methods. In the former method the mol. weight of each separate fraction is calculated from its intrinsic viscosity; in the latter this mol. weight is calculated as a differential from the properties of the preceding fraction.

Assuming that his method is also valid in the case of the natural rubber hydrocarbon, portions of the same latex were extracted simultaneously with a series of nine different mixtures of n-hexane, containing an increasing amount of isopropanol, for 48 hours (from 80/20 to 60/40 by volume). The average mol. weight of each extraction was determined by viscosity measurements using a viscosimeter of the Cannon-Fenske type at 35°C ± 0.05°. In calculating the mol. weight the Staudinger formula was used:

$$[\eta] = \lim_{c \to 0} \frac{\eta_{sp}}{c} = K\,M \qquad (1)$$

in which $[\eta]$ = the intrinsic viscosity
η_{sp} = the specific viscosity
K = a constant characteristic of a given polymer-solvent system
M = molecular weight

Staudinger (*J. prakt. Chem.* 157, 1940, 19) gives for rubber in toluene $K = 1.4 \times 10^{-4}$ in which the concentration is expressed in base mol. per litre. Calculated in grams per 100 ml this becomes

$$[\eta] = 2.06 \times 10^{5}\,M \qquad (2)$$

Since the constant K also depends upon the solvent we have determined this constant for every hexane-isopropanol mixture in the following way:

Masticated crepe is dissolved in these solvents and in toluene and viscosity measurements carried out at several concentrations. On extrapolating to zero concentration the intrinsic viscosity of the different solutions is found and using the formula of Staudinger, K of the solvent mixture is now calculated.

Fractionating Procedure

In 300 ml of the n-hexane-isopropanol mixture 3 ml of the diluted latex (1:1) is dissolved. After 48 hours the specific viscosity of the filtered solution at several concentrations is measured and after extrapolating to zero concentration the intrinsic visosity is found. Using the known K constant of each solvent mixture the average mol. weight of the dissolved rubber may be calculated. According to Sebrell the mol. weight of the separate fractions soluble in each solvent mixtures can now be calculated from these figures.

Preliminary Results

(a) It is found that the rubbers of the clones Avros 352 and LCB 1320 have the same plasticity and yet show quite different distribution curves and it is to be expected that this will offer an explanation of differences in performance of vulcanisates of these two clonal rubbers. Johnson (*Ind. Eng. Chem.* **40**, 1948, 351) studied the various mol. fractions of rubber and their behaviour during vulcanization and he concluded that those rubbers which had a large proportion of the low mol. weight fraction had poor qualities as to skid loss, resistance, tensile strength, etc.

(b) In clone Avros 385 occurs a fraction having a high mol. weight, notwithstanding its relatively good solubility. This implies a peculiar molecular structure, i.e. having side-chains or being crosslinked instead of the normal straight chain. It might be expected that the crosslinked molecular fractions contribute much to the low plasticity of this clonal rubber.

Paper No. 9.

The Rubber Hydrocarbon in Hevea Latex *

George F. Bloomfield †
(Rubber Research Institute of Malaya)

The average molecular weight of the rubber hydrocarbon in Hevea latex as it leaves the tree can be rapidly estimated by viscometric or osmotic measurements if the latex is brought into solution in benzene and the water phase removed by partial evaporation under reduced pressure. The use of a non-polar solvent such as benzene is preferred to the use of vistex solvents since a highly polar component in a mixed solvent can produce a coiling-up of the rubber molecule thereby reducing its intrinsic viscosity and the magnitude of this reduction is a function of the molecular weight and of the presence of gel structure. The molecular weight distribution, however, can be satisfactorily obtained by fractionation of vistex solutions prepared from latex as it leaves the tree.

The hydrocarbon in Hevea latex samples obtained at the tree is found to be of high average molecular weight with a broad molecular weight distribution from several million down to below 100,000 (estimated using Magat's values of the constants K and α in the Flory-Houwink equation $[\eta] = KM^{\alpha}$ and confirmed by osmotic measurements); the major portion of the hydrocarbon lies in the high molecular weight range with intrinsic viscosity in excess of 6 (mol. wt. above 10^6). The presence of small proportions (generally $<1\%$) of oxygen chemically combined with the rubber is confirmed in the most-soluble fractions of low mol. wt. (below 100,000) isolated in fractionation procedures, even when the latex is extracted from the tree under considerations precluding access of air. These are soft plastic rubbers which on treatment with alkali are converted into viscous cold-flowing gums without producing any appreciable change in their intrinsic viscosity.

Considerable differences in average mol. wt. are found between trees and although significant differences are also observed between days within given trees through-

† *Seconded from the British Rubber Producers' Research Association.*

* *This work is fully reported in* J. Rub. Res. Inst. Malaya, Vol. 13, Communication Nos. 271, 272 and 273.

out the course of a year's observations there are no indications of significant trends in mol. wt. during periods of biological activity such as refoliation.

Although in a number of cases intrinsic viscosity can be correlated with plasticity of different rubbers, there are such wide deviations from this correlation that intrinsic viscosity cannot be used as a reliable guide to plasticity. Pairs of hard and soft rubbers can be selected which have the same intrinsic viscosity, but osmotic measurements reveal considerable differences in mol. wt. The measurement of plasticity appears to be much more sensitive to the effect of structure (in the sense of regions of cross-linking) than is intrinsic viscosity. True microgel structure in which cross-linking is confined to the individual latex particles may in fact have little effect on the intrinsic viscosity while rendering the rubber very hard, and some experiments with benzidine hardening confirm this view. Although the hydrocarbon in fresh latex passes readily into solution on shaking, static diffusion into petroleum ether does in fact reveal the presence of microgel, the proportion of which is greater in hard rubbers than in soft ones.

Paper No. 10.

The Extraction of Latex under Sterile Conditions and some Properties of Sterile Latex

A. I. McMullen †

(Rubber Research Institute of Malaya)

An apparatus is described which enables the collection of fresh latex from the tree to be made under strictly aeseptic conditions and, if necessary, in the absence of light and air. The latex is also obtained free from possible contaminants originating in the bark. Certain trees have given as much as 150 ccs of such latex from a single stab (with the extractor knife) $1\frac{3}{4}$ inches long.

The behaviour of the resultant latex varies somewhat from tree to tree but in general it may be said that if undisturbed a yellow sediment appears at the bottom of the tube and occupies about one quarter of the total volume of latex. This yellow fraction may be re-dispersed by shaking and is liquid in the early stages. If the tube is continuously shaken the whole contents flocculate in a day or so. If the fractions are allowed to remain separate the yellow portion becomes flocculate after about a day and the white fraction stays quite liquid for weeks and remains uncoagulated indefinitely. The colour of the yellow portion decreases somewhat during what appears to be a coagulation process and coagulation of the yellow fraction is complete within several days. In the presence of a bi-valent metal sequestering agent this sedimentation of yellow material takes place more rapidly and the fraction remains completely liquid and sharply separated for much longer periods (several weeks). The pH of the samples after some months is in the region 6.0-7.0 and is often about 6.5. Absence of oxygen has no noticeable influence on the behaviour of the latex and no indication of oxygen uptake could be found in preliminary attempts to measure its respiration.

This behaviour in the complete absence of micro-organisms indicates that:

1. Spontaneous coagulation of *normal* fresh latex is due to the action of micro-organisms;

2. Preliminary coagulation is basically enzymic in nature and concerns only the yellow fraction material.

The photograph of the apparatus shows:

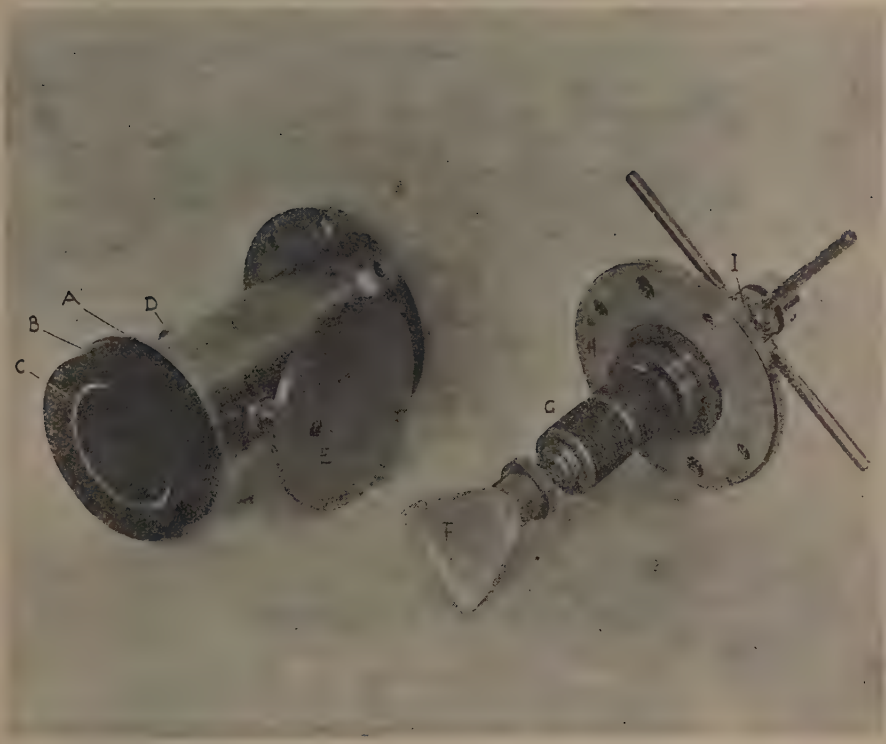
† *Seconded from the British Rubber Producers' Research Association.*

Stainless steel body with flange " A " which compresses the pure rubber gasket " B " against the prepared tree-trunk to effect a vacuum-tight seal. On further tightening of the apparatus to the tree by a " monkey-jack " clamping device (not shown) the steel body edge " C " is pulled slightly into the latex containing tissues to complete the seal. " D " is the outlet tube used for evacuation of the apparatus and filling with nitrogen when this is required. " E " is the delivery tube for the latex, attached to the flange which carries a vessel (not shown) for latex reception. The hollow-ground knife " F " is attached by means of a vacuum-tight metal bellows " G " to the flange " H ", which is fitted to the body flange, so that longitudinal displacement of the knife under airtight conditions is possible. " I " is a capstan bolt running on the threaded knife shaft, allowing a predetermined stab depth and easy extraction of the knife from the tree after tapping by straight insertion. The whole apparatus, apart from the metal bellows, is in stainless steel and the " pure " rubber washer compositions are formulated to withstand the necessary sterilization of the apparatus at 15 lb. steam pressure.

Discussion

The opinion was given that it would be most desirable to extend the work on respiration of sterile latex in more detail and attempt to ascertain if oxygen uptake really occurs with latex containing no micro-organisms and if so, whether the oxygen is taken up by the hydrocarbon or by the non-rubber material.

It was generally agreed that the obtaining of such latex free from micro-organisms and bark contaminants has opened the way to finding a solution to the mechanism of spontaneous coagulation.


A
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Paper No. 11.

The Contribution of the Yellow Fraction to the Viscosity of Latex

G. E. van Gils

(Indonesisch Instituut voor Rubber-Onderzoek)

The viscosity of latex is very important. However it is not possible to consider this as a specific property, characteristic of a certain latex, since it is very much influenced by the dry rubber content (DRC). Only if latices of the same DRC are compared, may the viscosity be an indication of the condition of the latex i.e. stability, composition, etc. We have tried to find a method to take the influence of the DRC into account.

Recently H. de Bruyn has derived a formula giving the relation between viscosity and concentration for concentrated emulsions, which agrees very well with the experimental data.

In the case of latex it is found that the data of Rhodes and Smith for centrifuged latices fit very well into the Bruyn formula but for field latex agreement is only attained if the DRC of the latex is "corrected" with a special factor—the "voluminosity."

"Voluminosity" is the ratio between the rheologically active volume of the dispersed phrase and the analytically estimated volume.

From the De Bruyn formula we have:—

$$\text{The Voluminosity} = V_R = \frac{0.8}{C_V}(1 - V\varphi_R)$$

C_V = volume of the rubber phase

$$\varphi_R = \text{relative fluidity} = \frac{1}{\text{relative viscosity}}$$

From a large number of experimental data, compiled by Mrs. De Haan-Homans, we have calculated V_R and found that this value is a clonal characteristic but is also influenced by seasonal variation.

In the second part of the paper the factors contributing to the V_R of latex are discussed. The most important factor is the presence of lutoid particles. By the addition of neutral salts or by increasing the pH of the latex the lutoids partly dissolve. This can also be studied by centrifuging the latex and measuring the volume of

the yellow fraction separated. The dissolution of a part of the yellow fraction by the addition of neutral salts indicates that this part is apparently composed of a complex coacervate.

In white fraction latex, where the lutoids have been discarded, the V_R values are smaller than in ordinary latex but still larger than 1.00. This may be due to solvatation or to an electroviscous effect. The experiment shows that increasing the pH (i.e. increasing the charge on the particles) increases the viscosity.

Paper No. 12.

Some Aspects of Theories of Spontaneous Coagulation

A. I. McMullen *

(Rubber Research Institute of Malaya)

Apart from the important technical questions concerned in the problem of the spontaneous breakdown of the Hevea latex colloidal suspension, the academic speculations arising in discussion of this interesting phenomenon have far-reaching implications in the study of plant physiology and have not yet been satisfactorily answered despite (or because of!) fifty years of spasmodic researches in the problem.

A brief description of the Colloid Chemist's picture of the organisation of molecules at the hydrocarbon-serum interface in Hevea latex is given with the corresponding theoretically possible methods of disorganisation of same, thus leading to flocculation and coagulation.

The causative agents of this disorganisation are regarded in the literature as being bacteria or enzymes and much controversy has existed as to which of these is essentially responsible. Much of the contradictory evidence is due to lack of uniformity in methods and material and partly to lack of differentiation between native enzymes and those originating in bacteria from external sources.

It is adduced from the reliable evidence available in the literature and from data supplied from research projects being carried out at the Rubber Research Institute of Malaya that spontaneous coagulation, as it is normally encountered, is due to the activity of contaminating micro-organisms, but that destabilisation of sterile latex or pre-coagulation of fresh latex is caused by an enzyme system set in motion by activators liberated from the lutoids present in the so-called "yellow fraction." The theory is advanced that the activating agent, most likely (on available evidence) Ca ion or Ca proteinate, is present in high concentration inside the lutoid "cell", or round the lutoid membrane. Such a concentration difference may be maintained in the latex vessels by normal biological dynamic equilibrium energy or by physio-chemical mechanisms as described by Danielli.

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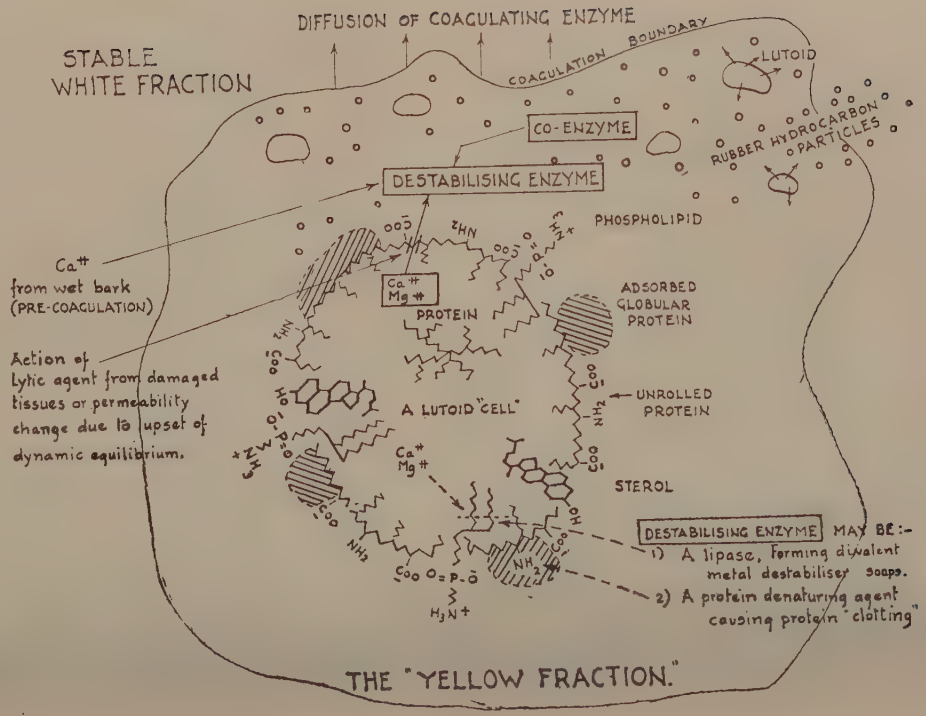
* *Seconded from the British Rubber Producers Research Association.*

When the latex is removed from this environment the activating ion is released into the serum in high concentration by change in membrane permeability due to breakdown of the equilibrium mentioned, or by the action of a lytic agent originating from damaged tissues or protoplasmic lining. The activator then unites with co-enzyme already present in the serum, giving rise to the active destabilising enzyme, in the neighbourhood of the lutoids. This enzyme then attacks the protein component of the protective layer surrounding the hydrocarbon particles by a denaturing action and flocculation occurs. This mechanism is illustrated in the schematic picture shown. (In this the structure of the lutoid is much simplified since surface techniques have shown that membranes behaving as osmotic barriers must be multi-molecular in thickness).

If the lutoids are allowed to settle under gravity then local or pre-coagulation takes place and complete destabilisation proceeds slowly as active enzyme or activator diffuses out from the coagulating centre. If the lutoids are maintained in a dispersed condition by shaking, total (but no fractional) coagulation occurs.

Discussion

It was suggested that possibly the explanation of such an apparently complex process as the spontaneous coagulation of sterile latex on the theory of a single mechanism may not represent the complete picture and that it might be necessary to consider that two influences are at work during disruption of the " mosaic " interfacial layers, namely a protein denaturing agent and a lipase producing long chain fatty acid from lipoid molecules.


DIFFUSION OF COAGULATING ENZYME
STABLE WHITE FRACTION
COAGULATION BOUNDARY
LUTOID
RUBBER HYDROCARBON PARTICLES
CO-ENZYME
DESTABILISING ENZYME
PHOSPHOLIPID
Ca++ from wet bark (PRE-COAGULATION)
Ca++ Mg++
PROTEIN
ADSORBED GLOBULAR PROTEIN
Action of lytic agent from damaged tissues or permeability change due to upset of dynamic equilibrium.
A LUTOID "CELL"
UNROLLED PROTEIN
STEROL
DESTABILISING ENZYME MAY BE:-
1) A lipase, forming divalent metal destabiliser soaps.
2) A protein denaturing agent causing protein "clotting"
THE "YELLOW FRACTION."

Paper No. 13.

The Lutoid Fraction and the Stability of Hevea Latex

A. S. Cook and A. I. McMullen *

(Rubber Research Institute of Malaya)

The discovery of the lutoid particles as a discrete phase in latex by Homans and van Gils and the subsequent separation of an unstable yellow fraction (YF) containing a large proportion of non-rubber substances has indicated the cause of the variation, among latices, in stability towards spontaneous coagulation. This has been confirmed by observations on sterile and variously preserved latices, in which the YF's preferentially flocculated and coagulated leaving white fractions which remained liquid for considerably longer periods.

Surface active material isolated from a YF by saponification was found to exhibit low interfacial tension (I.T.) against benzene and to give an I.T./concentration curve similar to that for potassium oleate.

Latices from different sources were fractionated and the distribution, between the fractions, of alcohol extractables, acids, nitrogen, ash, phosphorus and magnesium was similar to that obtained by Homans and van Gils. Potassium distribution was in a similar proportion.

The unstable latices had larger YF's which coagulated sooner than those from the stable latices. An unstable G.1 yellow fraction had a magnesium content more than twice that of the others and a P/Mg ratio of 0.8 compared with about 2.0 for the others.

Preferential coagulation of the YF can be facilitated, on the theory of van Gils, by the rapid and local formation of highly surface active divalent ion soaps of long chain fat acids. It is suggested that the level of instability in latex is due to the size and composition of the YF present, especially to the amounts of magnesium, long chain acids and the rate of hydrolysis of the lipids.

Discussion

Some observations were reported which suggested that there might be some connection between the amount of lutoid fraction and the incidence of brown bast.

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* *Seconded from the British Rubber Producers' Research Association.*

Paper No. 14.

Latex Stability

F. J. Paton

(Dunlop Malayan Estates Ltd. Research Centre, Malaya)

Glenshiel 1 is one of the best high yielding clones. The latex, however, is consistently unstable and has been selected for a study of the causes of variation in stability.

This latex has been shown to have a high magnesium content and a further study has demonstrated that it is largely associated with the serum. When ammonia is added to serum prepared by freezing, the magnesium is only partially precipitated in the form of magnesium ammonium phosphate, but on the addition of a further quantity of a soluble phosphate the magnesium is almost completely precipitated. The addition of ammonium phosphate to G.1 latex before centrifuging increases the stability of the concentrate. The effect reaches a maximum with the addition of 0.3-0.4% of phosphate. With a seedling latex of more normal properties substantially all the magnesium is precipitated from the serum when ammonia is added, indicating that there is sufficient phosphate available in relation to the magnesium.

The outstanding disadvantage of G.1 latex is that it does not give a satisfactory gel with sodium silicofluoride. It has been shown that when the serum is interchanged with seedling serum, normal gelling properties result. Normal gelling properties also result from the addition of phosphates as has been demonstrated by Madge.

G.1 latex has been proved to be more basic in character by electrometric titration of the acid latex. It is necessary to assume the existence of organic acidic substances in the serum, and this has been demonstrated by conductimetric methods. An analysis of the mineral constituents of G.1 serum shows that there is an insufficient number of acidic equivalents to balance the bases. The difference is stoichimetrically equal to the amount of acids found by titration. In the case of seedling serum there is an approximate equivalence between the acidic and basic groups in the ash. Conductimetric titration reveals only a very small quantity of organic acidic substances.

Paper No. 15.

Latex Stability and Composition

M. W. Philpott *et alia*

(Rubber Research Institute of Malaya)

Fertiliser treatments have been found to affect the level of mechanical and zinc oxide stability of latices from the plots of a replicated fertiliser trial (seedling rubber) in Field 6 of the R.R.I. Experiment Station. The application of phosphate and potash together has produced a notable increase in stability; nitrogen manuring appears to have had no effect. The experiment provides no information on the effect of applying P & K separately; nor is it certain that the observed effect of PK would be reproducible on other soils since the site of the trial is an exceptionally potash-deficient area.

To test the possibility that the effect of fertilisers on latex stability is correlated with a differential uptake of soil nutrients into the latex system, the plot latices were analysed for nitrogen, phosphorus, potassium and magnesium. Fertiliser treatments were shown to exercise a significant effect on the phosphorus and magnesium contents of the latex, PK manuring tending to raise phosphorus content and lower magnesium content. There is indication, too, that PK manuring raises the potassium content of the latex, although the effect is less obvious.

Effect of PK Manuring on Latex Stability and Composition

Treatment	Latex Composition, parts per 100,000 parts Latex Solids			Mechanical Stability (mins)
	Phosphorus	Potassium	Magnesium	
PK absent	98.5	407.5	69.0	8.57
PK present	113.5	434.0	58.5	12.21
s.e. of difference	± 4.2	± 12.9	± 2.7	± .81
Level of Significance of PK effect	< 1%	almost 5%	< 1%	< 0.1%

As a result of this fertiliser effect in Field 6 it is possible to establish marked negative correlation between

mechanical stability and the magnesium content of the latex (with or without constant phosphorus and potassium content), and marked positive correlation between stability and potassium content (with or without constant phosphorus and magnesium content). Mechanical stability is also positively correlated with phosphorus content but the relation appears to arise because of association between potassium, magnesium and phosphorus content. A significant positive correlation is also demonstrated between mechanical stability and the ratio of phosphorus magnesium content (with or without constant potassium content).

Following this investigation of latices from seedling rubber, an attempt was made to ascertain whether differences in the stability of clonal latices can be ascribed to differences in their mineral composition. Here it became apparent that other factors conditioning the stability may be present; for whereas latex of the notoriously 'unstable' clone Glenshiel I was found to have low phosphorus and high magnesium content (P/M generally less than 1.0), and latex of 'stable' clones such as P.B.186 and Pil. B.84 had relatively high phosphorus and low magnesium content (P/M_g generally greater than 1.5), latex of clone R.R.I.501 which has sub-normal stability, was found at two estates to have high P/M_g ratio (ca. 2.0).

These observations are tentatively explained by postulating an interaction between phosphate and magnesium ions in ammonia-preserved latex. If the amount of phosphate is insufficient to remove all the magnesium by complexing or precipitation (as in Glenshiel I latex) stability will be impaired by surplus magnesium ions remaining in solution. A considerable excess of phosphate will similarly destabilise the latex as a result of mutual interaction of phosphate anions in solution with charges on the surface of the latex particles. If the phosphorus and magnesium were wholly present in ionic form in the serum the weight ratio required for complete precipitation of both constituents as NH_4MgPO_4 would be 1.35. Thus latices with P/Mg ratio either less than or greatly in excess of some optimum value would tend to be unstable. Such a hypothesis would account plausibly for the observation that latices from plots of clone R.R.I.501 at the R.R.I. Experiment Station—all of which had high P/Mg ratio—exhibit a positive correlation for stability and magnesium content (P & K being constant).

Paper No. 16.

The Pre-Coagulation of Hevea Latex in Wet Weather

A. S. Cook and A. I. McMullen *

(Rubber Research Institute of Malaya)

In wet weather, excessive lump formation and clotting are found with certain latices, as is well known, and in many cases bactericides such as formaldehyde do not prevent this pre-coagulation. The cause can only be due to rain water, either by a dilution mechanism or by the introduction of bark substances into the latex.

It has been shown that dilution with distilled or rain water delayed the onset of spontaneous coagulation but that the addition of a cold water extract from the bark of *Hevea brasiliensis* very rapidly produced the flocculation of about 20% of the latex. This effect was obtained also when the extract was first sterilised, when the acid extract was made alkaline with caustic soda and when the latex contained small amounts of formalin. Sequestering agents did not altogether prevent this clotting.

The water extract of bark was found to consist mainly of carbohydrate and phlobotannin and one sample of dry extract contained 1.08% nitrogen, 3.66% phosphorus, 5.67% potassium, 0.65% magnesium and 1.18% calcium.

To confirm that bark substances play a part in pre-coagulation, latex was collected from wet trees, out of contact with the bark, by scraping this off and collecting over wax. Latex thus obtained remained liquid in corked tubes for about 30 hours after collection, compared with about 5 hours for latex collected through and over the bark.

Since sterile and alkaline bark extract caused the flocculation of latex it is unlikely that the presence of bacteria, enzymes or acids was responsible, and since carbohydrates would not produce acids by bacterial action in such a short time, it is suggested that pre-coagulation in wet weather is due to the contamination of latex with water-soluble bark substances, most probably the tannins but possibly also by the addition of certain enzyme activators to the latex. The mechanism of this pre-coagulation would seem to be the rapid flocculation of the

* *Seconded from the British Rubber Producers' Research Association.*

initial flowings or the yellow fraction either by precipitation and agglutination of the protective proteins by the tannins, or by the activation of the native latex destabilising enzymes.

Discussion

Many observations were reported by members present on the coagulation caused by the introduction of particles of bark into the latex and the tendency for certain clones, notably Avros 49, to have unstable first flowings and whose latices pre-coagulated when the trees were even slightly wet.

The effects of water on latex is obscure but it was mentioned that small amounts increased the viscosity of latex. In this connection it was pointed out that latex of Manihot (Ceara) is coagulated merely by the addition of water.

Paper No. 17.

Phosphatides of *Hevea brasiliensis*— Occurrence of Sugars

W. L. Resing

(Indonesisch Instituut voor Rubber-Onderzoek)

In the course of an investigation of the sugars occurring in latex from *Hevea brasiliensis* our attention was also drawn to the phosphatides present. Recently the phosphatides have gained considerable interest in connection with the variability of crude rubber, choline being a powerful accelerator for the process of vulcanisation[1]. Moreover the phosphatides have antioxidant properties and they appear to be an important part of the lutoids[2].

As long ago as 1922 Van Dillen[3] found galactose and glucose in the water soluble part of the latex. Quebrachitol is present in large quantities[4] and inositol derivatives have also been found[8]. The sugars may be important in connection with the metabolism of the rubber tree[5] as it is possible to synthesize rubber in vitro from carbohydrates in the presence of latex enzymes[14].

The phosphatides of latex have a high sugar content. Thus Rhodes and Bishop[6] estimated the amount of sugars, after hydrolysis of the crude phosphatides, to be some 10%. Tristram[7], purifying the phosphatides of *Hevea brasiliensis* and separating them into phosphatidic-acids and a lecithin fraction, found the sugar content of the latter to be 3.3%. According to him no appreciable amount of "cephalin" was present. Rhodes and Bishop supposed galactose to be the main constituent of their sugar fraction.

We have isolated the phosphatides from fresh unammoniated latex as described by Altman[8]. Latex was coagulated with an excess of alcohol, the coagulum thinly creped and extracted with alcohol in vacuo in a stream of CO_2. The alcohol extract was concentrated in vacuo and after extraction with ether, the phosphatides were isolated from the ethereal solution by means of acetone, redissolved in ether and reprecipitated with acetone. The phosphatides were separated into an alcohol insoluble "cephalin" fraction, and a "lecithin" fraction; these fractions showed the following characteristics:

"Cephalins"	Nitrogen	0.66%	"Lecithins"	Nitrogen	1.09%
	Phosphorus	2.52%		Phosphorus	3.22%

The cephalins were analysed for sugars. The sugar content, estimated with the reagent of Somogyi[9] after hydrolysis with $2N.H_2SO_4$, was 5.32%.

Hydrolysis of the cephalins with 0.01 $N.H_2SO_4$ for 12 hours at 100° and removal of H_2SO_4 with baryte yielded the sugar fraction.

The sugars present were detected by means of paper partition chromatography. As solvents we used butanol-ethanol-water (40-10-50)[11], ethylacetate-water-acetic acid (3:3:1) and ethylacetate-pyridine-water (2:2:1)[10].

Thus we found galactose, glucose and an oligosaccharide which, according to its place on the chromatogram, should be a trisaccharide; in the solvents used it runs somewhat slower than raffinose.

Arabinose, a constituent of the dissaccharide present in groundnut cephalin, could not be detected.

Further hydrolysis with $2N.H_2SO_4$ yielded a fraction containing sugars and polyhydric alcohols.

As the Rg* values of inositol and of the saccharide are almost the same (especially in B.E.W.), the chromatograms were run in duplicate. One was sprayed with a strong ammoniacal silver nitrate reagent[13], detecting both sugars and alcohols; the other was sprayed with aniline-trichloro-acetate, detecting the sugars only. As it could be possible that quebrachitol replaces inositol to some extent, we also estimated the Rg* value of quebrachitol, which was found to be 1.23 in ethylacetate-water-acetic acid (Rg of inositol 0.26).

We found inositol to be present and a sugar with an Rg value of 0.60 in E.W.A. (Rg value of sucrose in this solvent is 0.49).

Quebrachitol was not present. So far it has been possible to detect this substance only in the water-soluble fraction of latex.

In addition we were able to confirm that serine also occurs in cephalin from vegetable orgin[12], using B.E.W. as solvent.

Summarising the results we can say that the phosphatides of *Hevea brasiliensis* are linked to inositol and a carbohydrate-complex. The latter contains galactose, glucose and an oligosaccharide, probably a trisaccharide.

Quebrachitol could not be detected in the cephalins. Its Rg value in ethylacetate-water-acetic acid is 1.23.

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* *Rg value is obtained by dividing the distance the sugars have run by the distance moved by galactose.*

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Paper No. 18.

The Determination of the Inorganic Constituents of Hevea Latex

G. W. Drake

(Rubber Research Institute of Malaya)

Analytical methods used at the Rubber Research Institute of Malaya for the determination of the major inorganic constituents of latex are described. None of these is new but some of them have not previously been applied to the analysis of rubber total solids.

The ashing procedure is important. We agree with van der Bie that gentle ignition in an alkali medium, followed by digestion of the ash in acid solution is essential in the determination of phosphorus. We have shown that very slow ignition of an acidified rubber sample is required for the estimation of the metallic constituents.

The cobaltinitrite estimation of potassium has been shown to give correct results if precipitation takes place at a controlled temperature and an arbitrary conversion factor of 6.52 is used in the range 0.2 to 1.0 mgm. potassium.

The method of Drosdoff and Nearpass for magnesium has given consistent results provided the amount of magnesium present is in the higher part of the range (0 to 0.125 mgms. magnesium).

In the range of concentrations of calcium and magnesium found in rubber (Mg: Ca = 10:1) the oxalate method is satisfactory provided the calcium content is within the limits 1 to 5 mgms.

Paper No. 19.

The Amino Acid Constituents of the Protein of Hevea Latex

G. W. Drake

(Rubber Research Institute of Malaya)

The method of paper chromatographic analysis has been used to identify the constituent amino acids of the proteins in the latices of the three clones Glenshiel 1, R.R.I.501 and Prang Besar 186. Each latex gave an identical chromatogram comprising fourteen amino acids —alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, leucines, lysine, ornithine, phenyl alanine, proline, serine, tyrosine and valine, the relative amounts of which were approximately the same for each latex. No evidence of the presence of cysteine was found, although this acid has been shown by Tristram to be present in the protein of crepe.

Chromatographic analysis of the dialysate from latex serum indicated the presence in fresh latex of free amino acids, alanine, aspartic acid, glutamic acid, glycine, serine and the leucines, all of which were also identified in the protein.

The movement of the amino acids on the paper was found to be the same at 30° as that recorded by Dent at 20° when phenol-ammonia was the solvent, but to be not as great at 30° as at 20° when collidine-lutidine was the solvent.

Discussion

It was noted that the materials for the synthesis of protein were already present in the serum.

It was clear that the method would prove of value in the identification not only of amino acids but also of sugars and other non-rubber constituents.

Paper No. 20.

A Note on the Fractional Separation of Latex Proteins

B. C. Sekhar and A. I. McMullen *

(Rubber Research Institute of Malaya)

For a complete study of the influence of the native proteins and lipo-proteins on the stability of latex it is necessary to obtain these bodies in a pure unaltered form and to examine their colloidal and biochemical properties. To this end an attempt was made to separate, purify and classify as many discrete protein and lipo-protein fractions as possible.

Clear serum is obtained by freezing fresh latex in the field within half an hour of tapping and maintaining at-25°C for 16 days. On thawing, the serum may be expressed from the coagulum.

Several methods of separation have been tried—in each case the salt precipitation technique has been used, followed by dialysis and drying in vacuo. Oxidation and putrefactive difficulties have been encountered during the rather prolonged dialysis period.

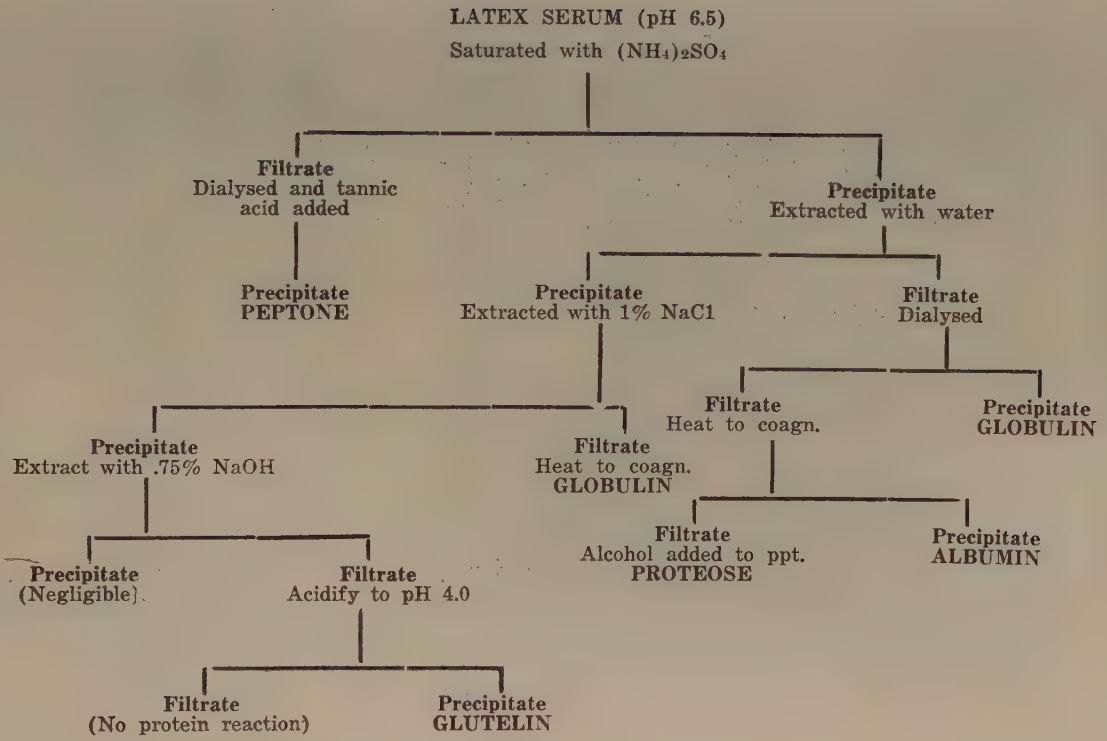
Albumins, globulins, proteoses and peptones have been shown to be present in serum and of these the albumin and globulin fractions occur in greatest proportions. The presence of glutelins as soluble complexes has also been indicated.

Analysis figures show that phosphorus (organic) is associated in undialysable form and in varying amounts with all fractions separated, and carbohydrate material is plentiful throughout, but is removable by washing and partial dialysis.

Tyrosinase has been demonstrated in association with an inhibitor which appears to be of protein nature.

The general form of the separation method being investigated at the present time is shown.

** Seconded from the British Rubber Producers' Research Association*


LATEX SERUM (pH 6.5)
Saturated with $(NH_4)_2SO_4$
Filtrate
Dialysed and tannic acid added
Precipitate
PEPTONE
Precipitate
Extracted with water
Precipitate
Extracted with 1% NaCl
Filtrate
Dialysed
Precipitate
Extract with .75% NaOH
Filtrate
Heat to coagn.
GLOBULIN
Filtrate
Heat to coagn.
Precipitate
GLOBULIN
Precipitate
(Negligible)
Filtrate
Acidify to pH 4.0
Filtrate
Alcohol added to ppt.
PROTEOSE
Precipitate
ALBUMIN
Filtrate
(No protein reaction)
Precipitate
GLUTELIN

Paper No. 21.

A Note on the Phosphatase in Latex *

Cheah S. L.

(Rubber Research Institute of Malaya)

1. The presence of phosphatase in latex is reported.

2. Phosphatase-active serum is obtained by coagulating fresh latex with dilute acetic acid. It was found that serum proteins precipitated by 0.35—0.50 saturation with ammonium sulphate possessed the highest activity indicating the presence of phosphatase in this fraction.

3. The method of determining the activity of phosphatase preparations is described.

4. The phosphatase activity is destroyed by drying at room temperature, and by the addition of ammonia. The enzyme is partially inhibited by Zn^{++}, Cd^{++}, Pb^{++} and CN^{-} ions, but not by iodoacetamide.

5. Disodium phenyl phosphate is to be preferred to sodium glycerophosphate as a substrate.

6. The optimal pH for latex phosphatase lies between 5.2 and 5.9 with acetate buffer and between 5.7 and 6.4 with citrate buffer.

7. Latex phosphatase possesses both hydrolytic and synthetic activity. But it is not active towards latex lipoid material, nor towards ethyl acetate and coconut oil. Apparently it plays no part in the break-down of latex lipoid constituents.

Discussion

Mrs. de Haan-Homans reported no significant differences in the rate of hydrolysis by latex phosphatase at temperatures of incubation ranging from 25°C to 45°C. No buffer was used in her work which was carried out in the region of pH 5.5. She obtained acid serum by coagulating fresh latex with formic acid.

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* *This work is fully reported in Part II of this publication.*

Paper No. 22.

The Particle Size of Oil-in-Water Emulsions

M. Van den Tempel

(Rubber-Stichting, Delft)

The papers No. 22 and No. 24 are abstracts from Communications 160 and 159 of the Rubber-Stichting (Delft), giving the first results of a long term fundamental research programme on latex stability, that has been started early in 1950 by Dr. Kraay, Mr. v. d. Tempel and co-workers under the direction of Dr. de Decker.

Determinations of the mean particle size in emulsified systems according to various methods yield values which can only be correlated with difficulty. Volume-average and surface-average radii are not equal, and their ratio depends upon the degree of asymmetry of the size-frequency distribution curve, $n = f(r)$.

Using an expression proposed by Dobrowsky to describe the size-frequency curve, equations have now been derived by means of which any of the important variables of an emulsified system can be calculated, if one of these variables and the volume-fraction of the emulsified oil (V) are known. Typical variables are: the total number of particles in a given volume (N); the volume-average radius (r_v); the surface-average radius (r_s); the most frequently occurring radius (r_m); the total interfacial surface (S).

The calculations can only be applied to emulsions of which the size-frequency curve can be described by the function of Dobrowsky, and of which at least one of the variables in known. In homogenized oil-emulsions and milk these conditions are not fulfilled, the degree of asymmetry of the size-frequency curve being too small.

The size-frequency distribution of Hevea Latex globules, as determined by Lucas, appears to follow exactly the course prescribed by the function of Dobrowsky. Application of the formulae leads to values of the variables which are, in general, in excellent agreement with other, independent determinations.

The work of the following authors can be correlated:

Lucas (1938)	.. $n = f(r)$ in Ultraviolet light;
van Gils (1941)	.. N in dark field;
Wren (1941)	.... r_v/r_s from thickness of monolayer
Hessels (1943)	.. $n = f(r)$ in visible light.

Thus we find the most frequently occurring particle radius to be 0.04 mμ, the volume-average radius 0.39 mμ and the surface-average radius of 0.23 mμ.

It is thought probable that on prolonged standing of an emulsion the size-frequency distribution acquires gradually a more asymmetrical shape, caused by coalescence of particles.

Discussion

Dr. Cockbain pointed out that Lucas' experiments again give a maximum in the particle size distribution curve at the limit of visibility (now for U.V. light), so that even this curve may be wrong; still smaller particles may be present and may be detected with the electron microscope.

Mr. Wren, Dr. v. Gils, Dr. Cockbain and Dr. Ruinen participated in a discussion on the interpretation of Mr. Wren's experiments on average particle size by means of "monolayer" thickness determinations. The main difficulties of the method must have been the following: (1) the "rheological" volume of the particles appears in this experiment, whereas the "optical" or "chemical" volume appears in other experiments. (2) The distribution of particles in the "monolayer" has only been observed in visible light and is therefore not definitely known.

Mr. Marsh and Dr. v. d. Bie contributed to a discussion of which the main points are:

(1) is the particle size distribution curve of young latex (in the tree) perhaps symmetrical ?
(2) is this distribution curve different in various clones and after various periods of ageing ?
(3) has the shape of this distribution curve a definite influence on creaming and centrifuging ?
(4) is it possible to study the former questions experimentally ? Dr. de Decker thought that (4) must be answered in the affirmative, and therefore answers to the other questions may be found.

Mr. v. Essen mentioned that the bigger particles consist of the lowest molecular weight rubber in Hevea latex (Hessels), whereas the opposite is true in G.R.S. latex.

Paper No. 23.

A Method of Determining the Specific Surface Area of Rubber in Latex

E. G. Cockbain

(British Rubber Producers' Research Association)

The method consists essentially in determining how much of a given soap can be adsorbed at the rubber/serum interface, using a suitable volume of latex of known D.R.C. Provided the area of rubber surface covered by one molecule of soap is known, the calculation of the specific surface area of the rubber (area per gram of rubber) can be carried out very simply. The method is not new and has been applied successfully in America for determining the specific surface area of polymers in the synthetic latices, *e.g.* G.R.S. latex. In G.R.S. latices both the composition and the concentration of the soap already present are known; all that is needed is to add more of the same soap (or a closely similar one) until no more is adsorbed as a monomolecular film. At this point soap micelles appear in the serum and can readily be detected by physical measurements. The concentration of soap in the serum at which micelle formation occurs is a characteristic of the soap and is well-known—hence the amount of soap adsorbed on the G.R.S. particles can be calculated.

In applying this method to natural latex, a number of difficulties must be overcome which are due to the complexity of latex. The chief difficulties are:—

(a) the composition and concentration of the naturally occurring soaps are seldom known with any accuracy.

(b) proteins are present which would be expected to interact with the soaps in solution.

(c) the method is complicated by the presence of inorganic salts.

Factors (b) and (c) can be circumvented by using a non-ionic soap as the surface active additive, while the influence of factor (a) should be eliminated by working at a pH value at which the surface activity of the naturally occurring soaps is greatly diminished. We have found it possible to obtain reproducible results by means of the following procedure:—

To a given volume of latex of known D.R.C., a known volume of a non-ionic soap solution of the Emulphor O type is added, followed by a small amount of polyvinyl alcohol solution as creaming agent. The latex is then acidified with acetic acid to a pH of 5.5, converting all the naturally occurring soaps to the free fatty acids which should be displaced from the rubber surface by the non-ionic soap. The latex is allowed to cream until a few c.c. of almost clear serum can be run off and the concentration of non-ionic soap in the serum is then determined by adding a solution of the dyestuff pinacyanol bromide. This dye undergoes a colour change from violet to pale blue when micelles of the non-ionic soap are present in solution. Knowing the total amount of non-ionic soap present, the amount adsorbed is obtained by difference. To obtain the specific surface area of the rubber, a value for the area of surface occupied by one molecule of the soap has to be estimated from physical chemical data. Further work with the method is in progress.

Paper No. 24.

Mechanism of the Gelling of Latex by Zinc Compounds *

G. M. Kraay and M. Van den Tempel
(Rubber-Stichting, Delft)

Zinc oxide is known to be more or less soluble in ammoniated Hevea latex. This solubility may be enhanced by several means, such as *e.g.* the addition of ammonium salts, or the action of trypsin. It is due to the formation of zinc-ammonia complexes.

The complexes themselves have no coagulating power, but when heated, zinc ions are split off by the complexes; these ions react chemically with the adsorbed soap on the rubber globules, forming an insoluble zinc soap and linking up all the rubber globules into a gel.

The mechanism can be represented by the following three reactions:

$ZnO + NH_3 +$ "water soluble acid" $\rightarrow$Zn-complex

Zn-complex (by heat etc.) $\rightarrow Zn^{\cdot\cdot} +$ .....

$Zn^{\cdot\cdot} +$ "fatty acid" $\rightarrow$insoluble Zn-soap.

Thus the "water soluble acids" and the "fatty acids" both play an essential but different part in the zinc oxide stability.

The mechanism could be proved by displacing the adsorbed natural fatty acids of the rubber particles by a soap which does not form an insoluble zinc soap, such as Igepon T. A latex treated in this manner does not gel when heated with zinc oxide and ammonium chloride at 70°C. When the Igepon T is displaced by ammonium oleate, the original fast gelation is recovered.

Depending on the concentration of zinc ions available at room temperature and the amount of fatty acids on the latex particles, thickening or even gelling may occur at room temperature.

The application of this new interpretation of the reaction mechanism may lead to new methods for the evaluation of latex.

Discussion

Dr. Cockbain asked whether there was any more definite proof of the facts that the complex as such is

* *This work will be reported shortly in the Transactions of the Institution of the Rubber Industry.*

not essential in the gelation and that $Zn^{\cdot\cdot}$ ions are existing at all in the pH range under consideration.

Dr. de Decker explained that these facts had indeed been proved more definitely by model experiments in which the essential compounds were brought together in water under appropriate conditions, omitting the latex itself. Complex and soluble fatty acid soap can remain in solution together; only under "gelation conditions" is the insoluble Zn soap seen to be formed, whereas under the same conditions the complex decomposes and yields $Zn^{\cdot\cdot}$ ions. Dr. v. d. Bie confirmed this by stating that no gelling at all could be obtained by using stable complexes.

Dr. v. Gils concluded that according to Dr. Kraay's hypothesis, it must be possible not only to find means for increasing the ZnO sensitivity of the latex, but also for decreasing it.

Mr. Schrieke pointed to the fact that the volatile acid determinations that have already been proposed in the literature (see also this Symposium) obtain a new significance in the light of Dr. Kraay's hypothesis.

Paper No. 25

A Chemical Stability Test for Latex *

J. H. Pidford
(H & C Latex Ltd.)

A method is proposed for evaluating the chemical stability of latex by determining its resistance to heating in the presence of zinc oxide. The method consists in mixing the latex with a standard zinc dispersion. The compounded latex is tested 1 hour and 24 hours after the zinc oxide addition. The apparatus comprises a jacketed sample tube with arrangements to flow through the jacket a mixture of hot and cold water to give any temperature desired. The sample of latex is drawn into the tube and held for one minute at the control temperature. The critical temperature, i.e. the highest temperature at which the compounded latex does not gel or coagulate, is recorded as the stability. Typical results are quoted, which illustrate the reproducibility of the test and the effect of certain variables.

The test was developed to evaluate latex for use in compounds which have to be stored and for use in preparing vulcanised latex, and is of definite practical value in this field. It is suggested that it may have wider applications and has been proposed as a simple test useful for incorporation into A.S.T.M. and similar specifications.

The work reported has been carried out by Mr. Coffin and his associates of the General Latex and Chemical Corpn. who do not, however, claim originality. They state "it is believed that this method was originally developed by the Firestone Tire & Rubber Co."

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* *This work is fully reported in Part II of this publication.*

Paper No. 26.

Studies on the Creaming of Latex and Synthetic Emulsions

E. G. Cockbain

(British Rubber Producers' Research Association)

In this communication attention is drawn to the basic analogy between aggregate creaming phenomena in latex and that in synthetic emulsions stabilised by pure soaps. Aggregate creaming is the term used when creaming results in the relatively rapid separation of serum and is characteristic of systems in which the primary dispersed particles are loosely aggregated; particle creaming refers to the much slower creaming of individual primary particles.

It is shown that aggregate creaming in emulsions stabilised by pure soaps commences at a soap concentration equal to, or slightly greater than, the critical micellar concentration *i.e.* the concentration at which aggregation of the soap molecules in solution occurs. Addition of inorganic salts, which affects the electric charge on the emulsion particles and, very probably, the degree of hydration of the adsorbed soap film, causes aggregate creaming to commence at a lower soap concentration which again corresponds closely to the critical micellar concentration of the soap (which is lowered by inorganic salts). The addition of water-soluble aliphatic alcohols also assists aggregate creaming but only insofar as these lower the critical micellar concentration of soap. It appears therefore that the essential condition for aggregate creaming in emulsions stabilised by soaps is the aggregation of the soap molecules themselves. This may be an essential requirement for all creaming agents, whether for latex or synthetic emulsions, as is suggested by the high viscosity of solutions of commercial creaming agents.

Paper No. 27.

Heat Sensitisation of Latex and Synthetic Emulsions by Polyvinyl Methyl Ether

E. G. Cockbain

(British Rubber Producers' Research Association)

Polyvinyl methyl ether (P.V.M.) is a water-soluble synthetic polymer of the composition

$$-CH_2-CH(OCH_3)-CH_2-CH(OCH_3)-CH_2-CH(OCH_3)-$$

When about 4% of the polymer (on the weight of rubber) is added to latex containing zinc oxide, the latex is rendered heat-sensitive provided its ammonia content is not too high. An advantage of the polymer is that it has no destabilising action on latex below about 35°C, heat-sensitisation setting in rapidly at this temperature. The mechanism of the heat-sensitisation process has been studied.

An aqueous solution of P.V.M. deposits polymer on warming to 30°-35°C, depending on the concentration, due probably to a dehydration of the polymer molecules. However, this precipitation does not itself result in gelation of latex when the latter is present. P.V.M. can be added to 60% ammoniated latex and although precipitation of the polymer will occur at approximately 35°C the latex does not gel. The reason can be found by studying the stability of synthetic oil emulsions stabilised by different soaps or proteins, in the presence of P.V.M. Provided the P.V.M. is more surface active than the stabiliser already present in the emulsion, it displaces the stabiliser from the surface of the emulsion particles. The adsorbed film of P.V.M. being highly hydrated, prevents coalescence of the emulsion particles at room temperature but the stabilising power of the dehydrated film is poor and the emulsion therefore breaks at about 35°C.

The fact that P.V.M. does not heat-sensitise a 60% latex at pH 10.5 in the absence of zinc oxide indicates that fatty acid soaps are present (as well as protein) at the surface of the rubber particles, since P.V.M. is sufficiently surface active to displace proteins from an interface but not fatty acid soaps. If the pH of the

latex is reduced, the stabilising efficiency of the adsorbed soap appears to be reduced, adsorption of P.V.M. occurs as before and at 35°C adhesion between the rubber particles occurs. In the absence of P.V.M., the same reduction of pH does not result in gelation of the latex at 35°C. The stabilising power of the naturally occurring soaps which remain undisplaced from the rubber surface by P.V.M. may be reduced by other means than a lowering of pH. One such means is reaction of the soaps with zinc ammonium salts, thus accounting for the gelation of 60% ammoniated latex – zinc oxide, in the presence of P.V.M. at 35°C.

Paper No. 28.

Correlations of some Constituents of Latex and Technical Properties of the Rubber *

P. Compagnon and C. Cretin

(Institut des Recherches sur le Caoutchouc en Indochine)

Systematic analysis done on latex from different sources (various clones, 8 and 15 years old) shows that copper and nitrogen contents and DRC are correlated.

Correlation coefficients have been calculated:—

—between Copper content and DRC and also TS

—between nitrogen content and DRC and also TS

—between copper content and nitrogen content

These results are discussed with reservations since the analysis concerned only one part of the year, and all the latices examined came from the one area (Laikhe Station). It may be that these correlations are only valuable under certain seasonal and ecological conditions.

These results are related to the technical properties of the rubber from the latex, because certain relationships have been found between nitrogen and modulus, and between the DRC of field latex and the modulus. They also bear some relation to the physiology of latex and to the experiments on tree injection with copper sulphate, in which have been found:—a slight increase in the copper content of the latex, a lowering of the DRC, an increase in the modulus of the resultant rubber and it would seem, according to the first analysis made, an increase in the nitrogen content.

** This work is fully reported in Part II of this publication.*

Paper No. 29.

The Preservation of Latex at Neutral pH

A. I. McMullen *

(Rubber Research Institute of Malaya)

This programme of research was carried out on the assumption, indicated by available evidence, that destabilisation of fresh latex is due to bacterial or enzymic action and in the mechanism of this action it is possible that di-valent ions are important. It was required, primarily, that the resultant preserved latex have a pH close to 7.0.

With this in view a large number of bactericides, enzyme poisons, emulsion stabilisers and ion sequestering agents, and mixtures of these, were added to fresh latex and the resultant preservation observed.

Some of the observations made are as follows:

(a) Complete bulk coagulation rarely occurs and initial coagulation of the "yellow" fraction takes place in a large number of cases, the remaining latex maintaining its liquid form for a very long time. It is shown that removal of the yellow fraction enhances preservation of the white fraction.

(b) Di-valent ion sequestering agents, at high concentration, can maintain latex in a fluid condition for long periods of time.

(c) Normally efficient bactericides and emulsifiers, if ionic in nature, are very inefficient in latex. This was shown to be due, in many cases, to interaction with the serum proteins.

(d) An approximately reciprocal relationship exists between the preservative power of stabiliser and biological inhibitor in mixtures of the two.

(e) Non-ionic stabilisers are the most effective, especially above a "critical concentration" of about 0.25% on the latex and in the presence of a good bactericide.

(f) Latices stabilised with non-ionic ethylene oxide polymers are heat-sensitive and the sensitivity may be adjusted over a consider-

* *Seconded from the British Rubber Producers' Research Association.*

able range of temperature by suitable addition of salts.

(g) Samples of latex have been maintained in completely liquid and undecomposed condition for 12 months at pH 6.5.

(h) A major disadvantage of the process of preservation of latex by non-ionic materials is the consequent difficulty of producing coagulation when required.

Most of these phenomena can be explained on the basis of the observation (a) that complete coagulation due to bacterial action only takes place after a certain amount of pre-coagulation has occurred. It is known that such coagulation concerns the lutoid fraction and the theory that a specific protein denaturing enzyme native to the lutoid is responsible has been put forward elsewhere. This theory may also be used to explain the observations made in this work. The possibility that "neutralization" by acid of some protein of high iso-electric pH present in high concentration at the lutoid interface is the cause of pre-coagulation (as suggested by the fractional coagulation procedure used to obtain pale crepe), is rather negatived by the observation (b) unless the bacterial enzyme which produces acid is dependent on divalent ions.

On this view the action of soap and the approximate reciprocal relationship observed between the preservative power of soap and enzyme-inhibitor in mixtures of these agents might be explicable on the basis of progressive replacement of the specific protein by soap molecules and consequent reduction in inhibitor requirement.

Discussion

It was suggested that the extra expense usually associated with preservatives of the types used in this work may be considerably reduced by using concentrated latices in which the anti-bactericide effect of the serum proteins would be considerably diminished thus enabling smaller concentrations of reagent to be equally effective.

Paper No. 30.

Studies on the Temporary Preservation of Hevea Latex

A. S. Cook

(Rubber Research Institute of Malaya)

The importance of producing uniform sheet rubber, with the consequent transportation of field latex considerable distances to central factories where bulking is required on a large scale, has dictated the need to preserve latex for several hours or a few days. The usual latex preservative, ammonia, is expensive and is not very effective is small amounts.

Formalin has been found to be an adequate preservative for normal latex; 0.1% HCHO in the latex giving smoked sheet of first grade quality when coagulated the day after addition, without affecting the preparational methods nor the technical properties of the rubber to any marked extent. For longer preservation, the presence in formalin latex of salts and mild alkalis such as ammonium or sodium hydroxides, carbonates, phosphates or silicates, some of which do not preserve latex, greatly increase the latex stability. One sample of latex containing 0.1% formaldehyde and 0.1% sodium metasilicate remained in a liquid condition for about 40 days. In many cases a yellow fraction flocculated and settled out before complete coagulation.

Formaldehyde does not disperse the lutoids and therefore enables the yellow fraction to be separated in a liquid condition. The presence of salts does not prevent the separation, provided that the pH is not raised much above 7. Silicates give a large viscous yellow fraction. These facts should prove useful for the preparation of pure uniform concentrated latex containing few non-rubber substances.

Since formaldehyde is a bactericide and enzyme killer and reacts as a tanning agent with proteins, the mechanism of preservation is probably the prevention of acid formation and lipid degradation; coagulation taking place by bacterial action after the formaldehyde has reacted with the proteins. Salts may enhance the stability by (a) buffering the pH, (b) sequestering the

divalent ions in the latex, and (c) in the case of silicates, perhaps by the formation of strong complex films with proteins at the surface of the rubber particles.

Discussion

It was reported that in many areas, especially in Java, as much as 0.2% HCHO alone did not preserve latex and it was suggested that the cause might be the high level of magnesium found in these areas.

Paper No. 31.

The Determination of Volatile Acids in Latex

M. W. Philpott and K. C. Sekar
(Rubber Research Institute of Malaya)

A common method of assessing deterioration in natural rubber latex is to determine the total concentration of acidic substances by titration with KOH (Jordan, *Proc. R. Tech. Conf.* 1938, p.111). The method suffers from several disadvantages both as a test for decomposition and as an index of processing behaviour. First, it estimates all acids which titrate up to pH 11.2 and thus fails to discriminate between stabilising (*e.g.* soap forming) acids and destabilising (*e.g.* formic, acetic etc.) acids. Secondly it includes conjugate acids of bases, basic groups of zwitter-ions from amino acids etc. Thirdly it fails with latices to which soaps or fixed alkalis have been added.

It has now been shown that a substantial proportion of the acids formed in latex as a result of natural degradation processes can be distilled in steam and that these volatile acids consist mainly of acetic with some formic and propionic acid. It is considered that a test of spoilage based on the estimation of these acids would be free from the disadvantages which are inherent in the KOH titration, and a method of determining quantitatively the volatile acids in latex has accordingly been worked out.

The method is based on the principle (cf. Young, "*Distillation Principles and Processes*", 1922, p.289; also Dyer, J. *Biol. Chem.*, 1917, 28, 445) that when a dilute solution of a volatile substance is distilled *at constant volume* the amount of volatile solute passing over into the distillate is related to the volume of the distillate by

$$K = \frac{1}{v} \ln \frac{a}{a - x} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

where a is the amount of solute originally present in the distillation flask, x is the amount which passes over into volume V of distillate and K is a constant characteristic of the volatile substance.

Experiment has shown that the distillation constant K is not wholly independent of the dimensions of the distillation apparatus and the conditions of the distillation; nevertheless consistent and reproducible results can be obtained if the apparatus is not varied and the procedure is adequately controlled.

Outline of Method

50 gms. of latex of known DRC is diluted with 100 ml. of water and coagulated with 5N H_2SO_4 (20 ml.) plus 20% phosphotungstic acid (5 ml.) to precipitate serum proteins. The serum is pressed out of the coagulum and filtered, and a known aliquot of the total aqueous phase is distilled with steam (Fig. 1), keeping the volume constant and controlling the rate of distillation at about 200 ml. per hour. The first 50 ml. and the next 200 ml. of distillate are separately collected and titrated with 0.01N NaOH. Formic acid is determined in the combined distillates by any convenient method.

From the titres ($^{t}50$ and $^{t}200$) of the successive distillates, the previously determined distillation constants for formic, acetic and propionic acids, and the known amount of formic acid in the distillate it is possible to calculate the amounts of formic, acetic and propionic acids initially present in the distillation flask, and hence in the sample of latex.

In practice it frequently happens that the ratio of the two titres ($^{t}200/\ ^{t}50$) approximates to the ratio which is characteristic for acetic acid (ca. 2.5); it may then be sufficiently accurate to assume that the volatile acid consists exclusively of acetic acid. The determination of formic acid is then omitted and the total volatile acid, as acetic acid, is calculated from

$$^{t}50 + {}^{t}200 = \beta A$$

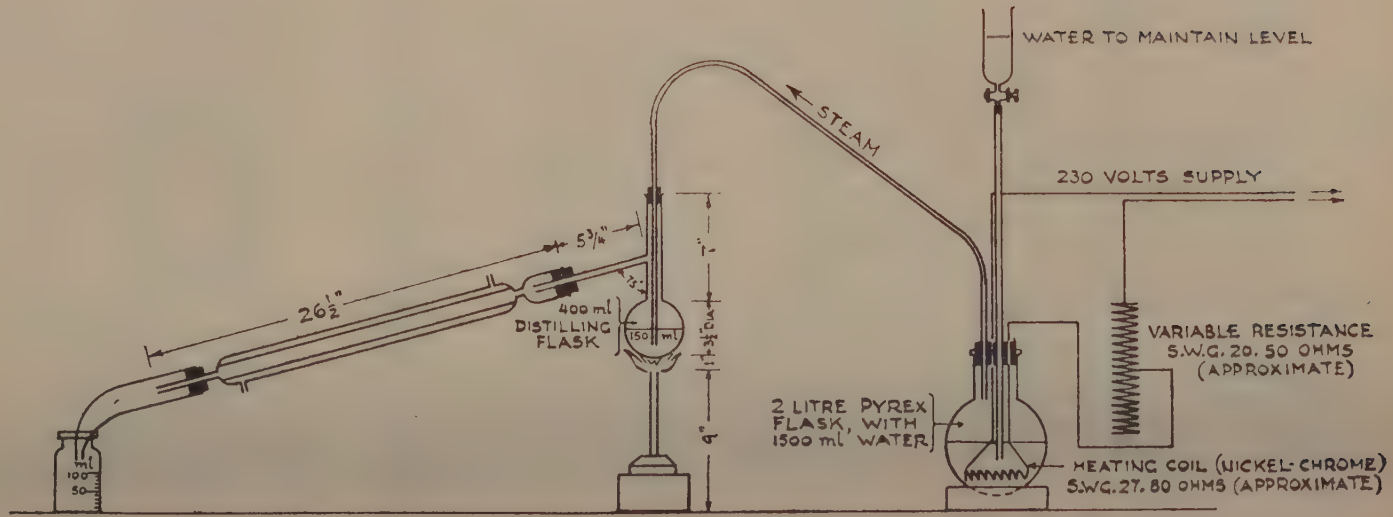
where β is a constant characteristic of acetic acid and the distillation conditions (approx. 0.6) and A is the amount of acid initially present in the distillation flask.

Application

The sensitivity of the test for diagnosing incipient decomposition in latex is illustrated by the typical observation that in the course of 8 days' storage the properties of an inadequately preserved latex changed by a factor of 2 for conductivity, 3 for KOH No. and 30 for volatile acid content.

FIG. 1

DETERMINATION OF VOLATILE ACIDS IN LATEX



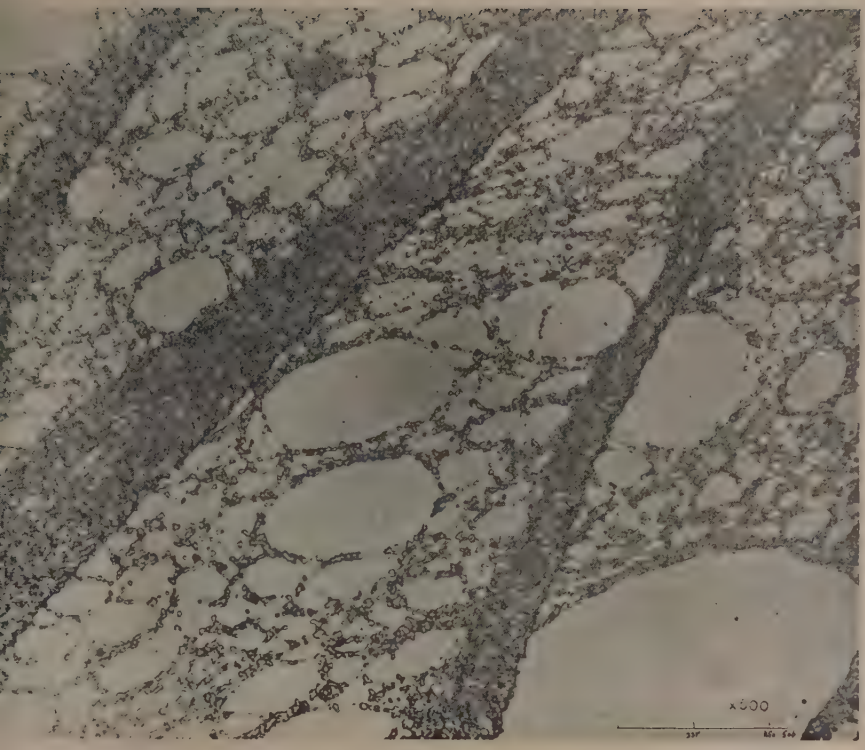


Fig. 1

Fig. 2

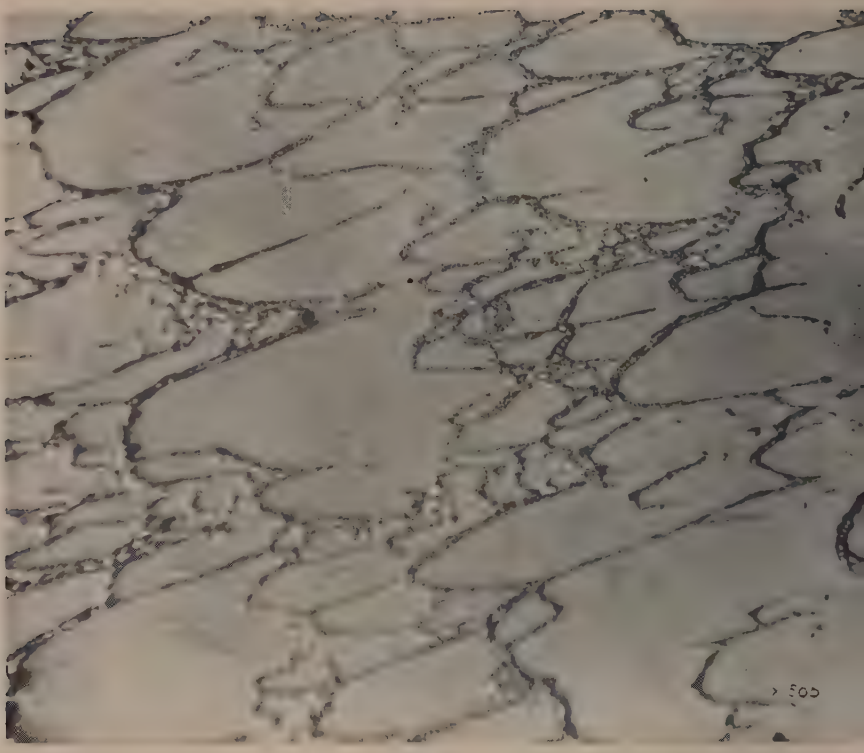


Fig. 3

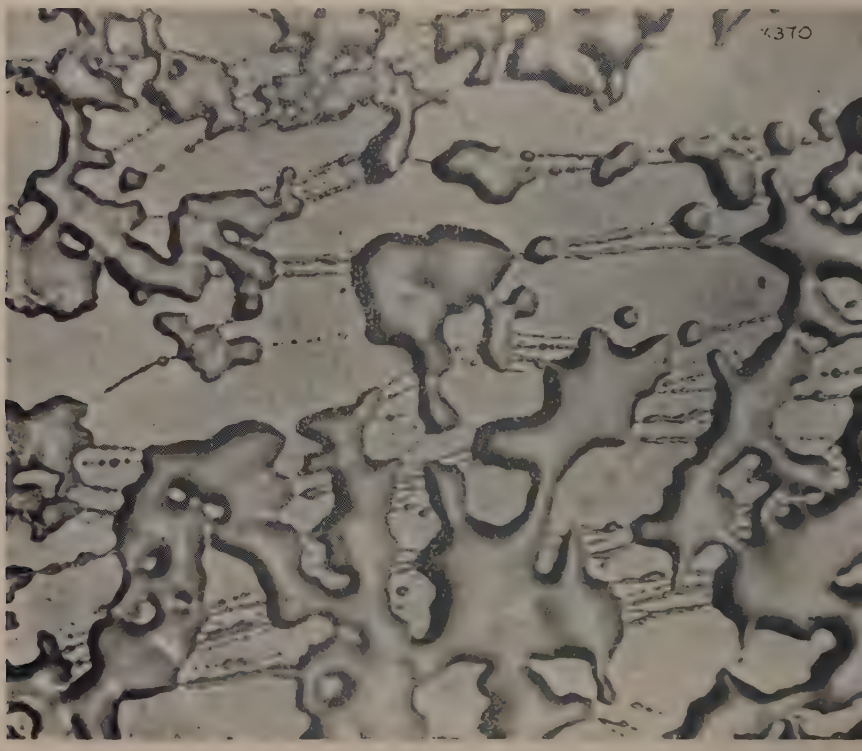


Fig. 4

Fig. 5 (wet)

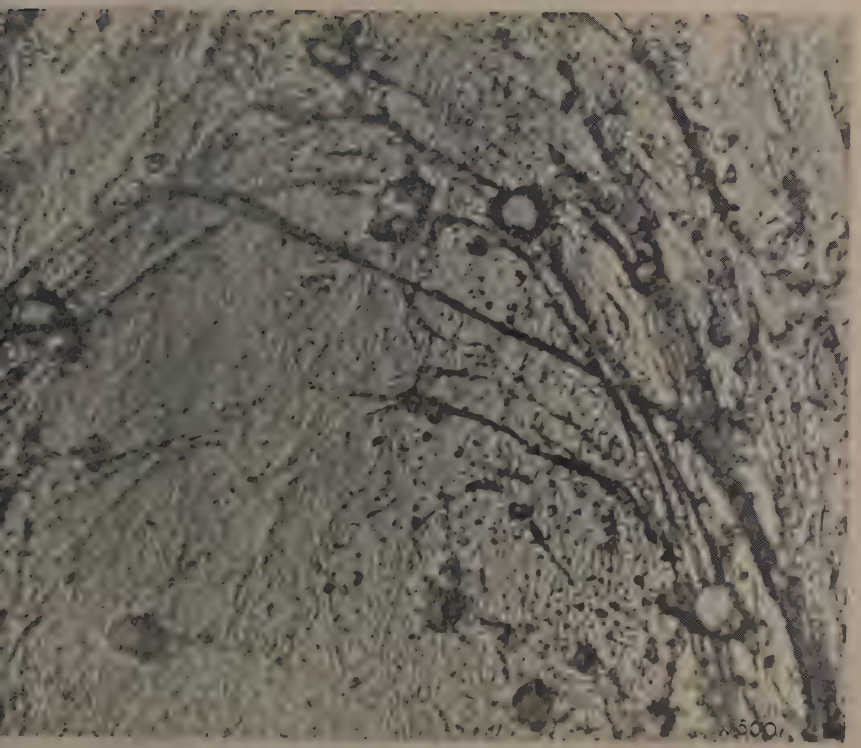


Fig. 6 (dry)

Paper No. 32.

The Structure and Strength of Fresh Coagula

A. I. McMullen *
(Rubber Research Institute of Malaya)

The point of transition from rubber hydrocarbon in the form of particles in latex to that in the form of solid rubber where the hydrocarbon is the continuous phase has always been a matter of speculation. It is not the claim of this short paper to have decided the issue but some of the observations made herein indicate certain lines of approach that may be made the subject of further, more intensive study.

In the micro investigation of the coagulation procedure monoparticle thick films of latex have been spread on coagulating media by the Lanmuir-Adam technique, removed on microscope cover slips after various treatments and examined at high magnifications. Corresponding bulk measurements of tear strength and tensile strength have been made in an approximate way.

The micro investigation shows that the fresh coagulum, as obtained under normal acid coagulation conditions, is particulate in nature but is quite coherent (Fig. 1). On standing, or on stretching (Fig. 2) or rubbing gently (Fig. 3), the particles become less discrete and ultimately a mosaic hydrocarbon continuum network is established which is readily transformed into a solid sheet on further treatment. Prolonged storage of this film leads to breakdown and liquefaction of the rubber (Fig. 4). It is suggested that local displacement of protective protein occurs in the initial stages with consequent local hydrocarbon links, the system being thermodynamically unstable.

The twin photos (Fig 5 and 6) show that even air-dried yellow fraction latex film exhibits a mosaic or particulate structure when wet but a continuous structure when dried completely under high vacuum.

The strength of the bulk coagulum depends mainly on the dry rubber content, rather than on the non-rubbers. The addition of various protein dispersing reagents to the latex has a noticeable influence on the tensile strengths and drying rates however; most additions decreasing the strength and several (such as urea, saponin) greatly accelerating the loss of moisture during the latter period of drying.

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* *Seconded from The British Rubber Producers' Research Association.*

PART II

COMPLETE PAPERS

Paper No. 2.

Plant Hormones and Yield in *Hevea Brasiliensis*

G. W. Chapman

(Chemara Central Research Station, Malaya)

Introduction

This work, (which falls into three phases) was started in 1937 because cowdung mixtures applied to the scraped bark to improve bark renewal and yield were widely used by Asian planters.

In the early work, bark analysis was carried out as well as the application of mixtures to the bark. These mixtures included numerous combinations of various oils —both vegetable and mineral—cowdung, plant nutrients and sulphur in various forms. The bark analysis is described below, the bark treatment experiments may be dismissed with the statement of two important conclusions.

1. All the better mixtures had one common factor, they contained plant hormones.
2. Elementary sulphur was highly beneficial under certain conditions, when clearly (as judged by the smell) some sulphur compound was formed.

Other work in Malaya (1) and Ceylon (2) belonging to this phase has also shown that yield can be increased by applying certain vegetable oils to the bark, preferably after scraping.

The second phase of this work commenced after the war when selective weed-killers and other synthetic plant hormones were available in commercial quantities. Work was then started with α-naphthalene acetic acid, 2:4-Dichlorophenoxy acetic acid and its methyl derivative marketed as Methoxone.

In the course of this work more than 50 different preparations were tried on the bark of rubber trees. Broadly speaking they all gave the same type of results but some gave much better yield increases than others and 2:4-D proved the most satisfactory of the three hormones.

These results clearly suggested the hypothesis that natural hormones played a part in the yield capacity of rubber trees. Thus the work entered its third phase with the development of an analytical method for estimating hormones in latex, sufficiently rapid for routine work.

A preliminary survey of the conditions causing variations in the hormone content of latex has been carried out to determine whether yield is influenced by hormone production in the tree, but much work remains to be done.

Effect on Yield of Applying 2:4-D to the Bark

Initially, old seedling rubber was used and each treatment was applied to 30 trees. Before treatment some hundreds of trees were individually yield recorded by the cup coagulation method and the few trees which showed erratic yields were rejected. The remainder were then graded on yield so that every treatment contained three equivalent groups of 10 trees each. Group I contained the best yielders and group III the worst. Later when more uniform young seedling and budded rubber were used the number of trees in each group was halved.

The most outstanding feature of these experiments is the better response of the trees in groups II and III compared with group I. This is observed both for seedlings and buddings, but as might be expected the difference is greater for seedlings than buddings as is seen from three experiments which are described below. In these experiments the outer dead zone of the bark was removed by light scraping over a region 3 inches below the half spiral tapping cut.

On estate 'A' on young seedling rubber there were 10 treatments and in all except control the bark was scraped and painted with nine different preparations containing 2:4-D. On estate 'B' the following six clones were treated with the standard preparation used on the Guthrie Group of estates: G.1, B.D.5, Tj.1, P.B.25, Pil.B.84 and P.B.86, and on estate 'C' the following three clones were treated with the same preparation: Tj.1, P.B.25 and P.B.86. In the figures given below as percentage of control the averages for all treatments or clones are given by groups for the first month after treatment.

	Group I	Group II	Group III
Estate 'A'—Seedlings ..	152	162	193
„ 'B'—6 clones ..	129	142	157
„ 'C'—3 clones ..	179	189	212

In all the above cases group III is significantly greater than group I, but this is not always so for the difference between group I and group II. On estate 'A' the yield

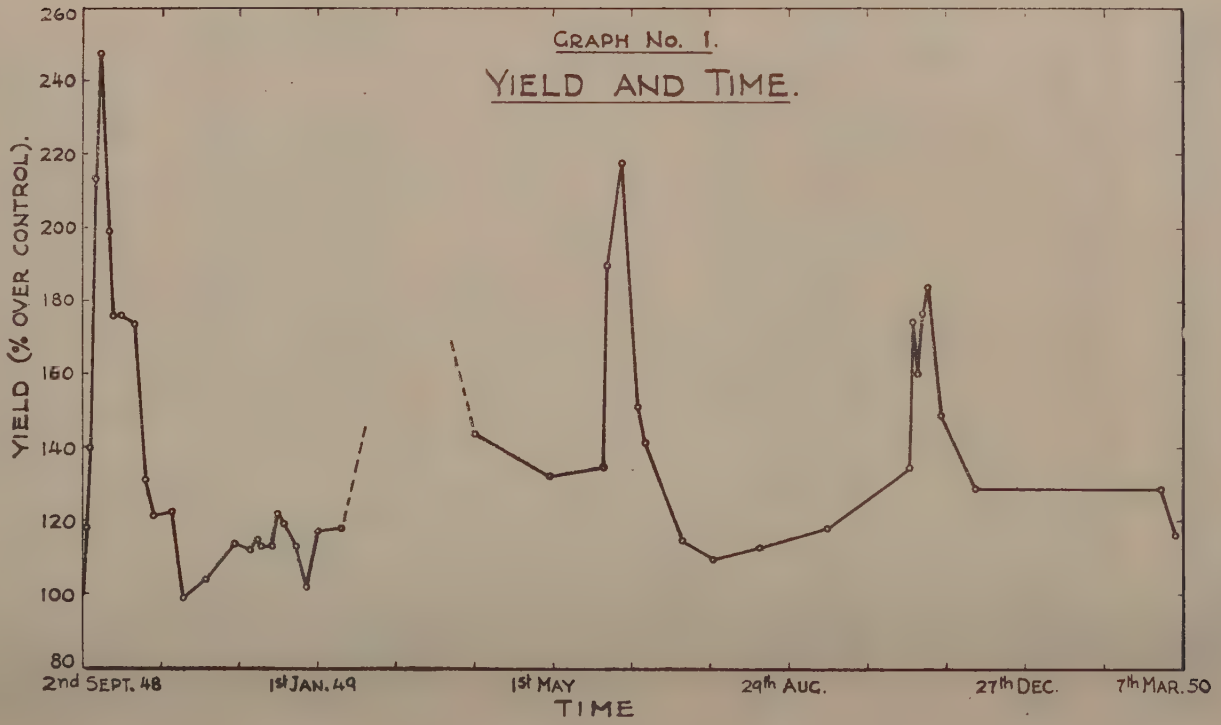
ratios for groups III, II and I were approximately as 1:2:3. With the clones the differences in response between groups were less as was also the case with seedlings when the yield differences between groups were less.

The yield responses varied considerably between different clones on the same estate and between the same clone on different estates as the following extreme figures illustrate:—

	Estate 'B'			Estate 'C'		
	Group I	Group II	Group III	Group I	Group II	Group III
P.B. ..	95	119	130	155	157	169
Tj.1 ..	154	168	204	237	252	288

Other experiments demonstrated the following facts:

1. Scraping the bark even without other treatment usually raises the yield temporarily, but afterwards the yield falls below the control.
2. All the preparations used were more effective if applied to the scraped bark, presumably because absorption was facilitated.
3. Full spiral gave a good increase, but the dripping often lasted till next day at the peak yield period.
4. Late dripping is a characteristic of all hormone treatments at the yield peak. Indeed the increased yield is largely due to a longer dripping period.
5. Despite the late dripping, D.R.C. was only slightly lowered by hormone treatment, normally only 2 or 3%.
6. The extent and the position of the area treated was of primary importance. If applied only above the tapping cut yield is actually depressed, while if applied both above and below a slight increase is obtained. Applying over a 3″ band below the tapping cut appears to be sufficient to give a maximum increase. It is better to treat a narrow band than to treat a larger area because the treatment can be repeated as soon as the treated bark has been tapped away.
7. Trees on daily or slaughter tapping systems do not react well to treatment with hormone preparations and should not be treated with them.
8. The curve showing the relationship of yield to time after application is characteristic and is shown in Graph (1). Maximum yield is attained within a week to 10 days. Thereafter yield falls


GRAPH No. 1.
YIELD AND TIME.
YIELD (% OVER CONTROL).
260
240
220
200
180
160
140
120
100
80
2nd SEPT. 48
1st JAN. 49
1st MAY
29th AUG.
27th DEC.
7th MAR. 50
TIME

steadily until 6 weeks after the application and then rises to a second maximum at the end of about 2 months. From then on it falls slowly over a period of months but never seems to drop below control. Numerous experiments have shown that the yield remains a few percent above control for a long time after all the treated bark has been removed. Subsequent applications after about 4 months do not produce results identical with the first, the initial maximum is not so high, but the minimum and second maximum are both higher so that the yield obtained over the later 4 months periods are the same as that over the first 4 months. This statement can only be made for P.B.86 as no other clone has yet been treated four times.

A count of dry trees showed no significant difference between the treated trees and the controls, nor did the trees show any other sign of injury. Nevertheless in the present state of our knowledge it would be a risk to treat high yielding trees at frequent intervals over a prolonged period.

At present it is suggested that treatment should not be more frequent than once in four months and the wintering period should be avoided. In some area Tj.1 latex has shown an increased tendency towards precoagulation with treatment at the wintering period and apart from this consideration a tree in the process of refoliation should not be heavily tapped.

Under certain conditions the hormone treatment should not be used. P.B.25 as reported above sometimes gives a very poor response but otherwise there appears to be no reason for not treating it. But Lunderston N and B.D.5 alone of the clones tested should never be treated as the bark is injured to some extent although no permanent damage is done to the trees. Damage can also be caused by very deep scraping combined with excessive application of the oil. Old trees with very thin and knotted bark cannot be treated because of the impossibility of scraping such trees.

Relation of Bark Composition to Yield

In order to obtain an insight into the mode of action of hormones both bark and latex have been analysed.

The work on bark was incomplete when interrupted by the Invasion.

The survey of composition gradients in bark was carried out for the major nutrients nitrogen, phosphorous, potash, calcium and magnesium. All nutrients showed marked changes in a radial direction when the bark was divided into three zones. The first or innermost zone is soft and is not removed in normal tapping. The second zone is the latex yielding region and the third is brittle and contains many stone cells. The outer dead layer was discarded.

Bark composition changes in a vertical direction were determined from 20″ to 120″ above the ground, both for virgin bark and for trees in tapping. No consistent difference was found between high and low yielding trees in the change of bark composition with height. However, in all the comparable groups examined the bark of the higher yielding trees was higher in calcium, particularly in zone 1.

The effect on bark composition of treatment with the natural hormone mixtures then in use was examined. Bark magnesium was invariably increased but there was no consistent effect on the other nutrients unless they were present in the preparations applied to the bark, when they were apparently absorbed. Whether it is a mere coincidence that the divalent metals are higher in both natural high yielders and those produced artificially cannot be stated, but it is well known that the ratio of divalent to monovalent metals in tissues affects their permeability and might therefore have a bearing on yield.

Estimation of Natural Hormones in Latex

In the determination of hormones in latex the standard methods given by Jensen (3) and Went and Thimann (4) for the estimation of auxin were used with modifications.

The routine procedure used for determining the hormone in latex was as follows:—

200 cc. of latex were diluted with 100 cc. of water, warmed to 40°C and coagulated with 30 cc. of 2% phosphoric acid. 250 cc. of the expressed serum was saturated with acid sodium phosphate and shaken for half an hour with 3 cc. of methyl isobutyl carbinol. The gelatinous precipitate was filtered off on a Buchner through

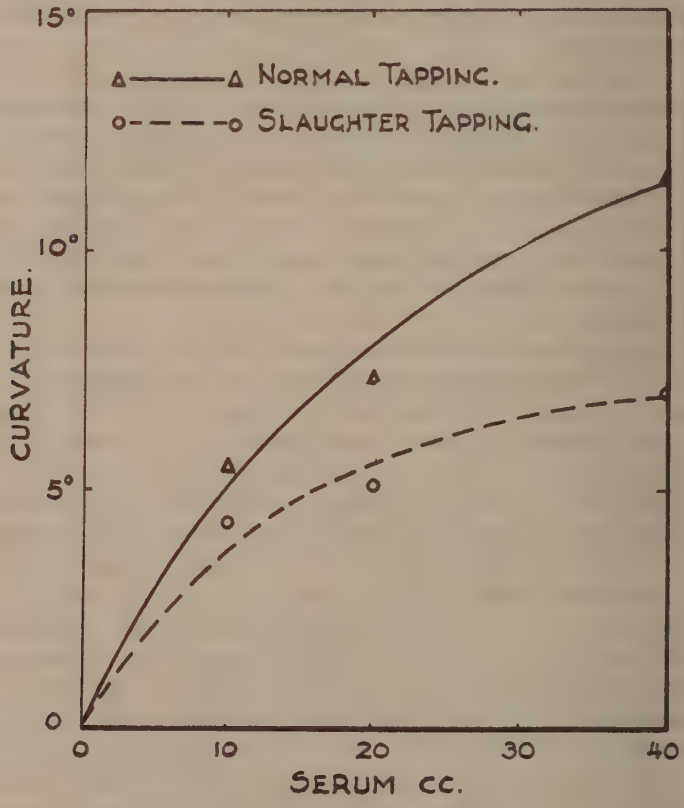
asbestos and the filtrate extracted four times with 15 cc. of the above alcohol. The sludge was centrifuged out and the alcohol was extracted three times with sodium bicarbonate solution, to give 20 cc. of extract. This was saturated with acid sodium phosphate and acidified with phosphoric acid to pH 3 before extracting four times with 1/6 volume peroxide-free ether each time. The ether was evaporated and the almost invisible residue dried in the vacuum desiccator over caustic soda for 12 hours. The residue was then taken up in a measured volume of 90% alcohol and filtered through a Gooch crucible. The alcohol was evaporated off after division for testing had been made.

Testing was carried out by the Avena method as standardised by Went (4), and curvatures were recorded photographically after $2\frac{1}{2}$ hours. The hormone concentrations are expressed in terms of the equivalent weight of indoleacetic acid in γ per litre of serum. When curvatures are given they are corrected to an assumed curvature of 15° for 20 γ per litre indole acetic acid. The serum is true serum calculated free from diluting water, and acid phosphate.

In all tests agar blocks were prepared from 0.5 cc. of concentrate mixed with 0.5 cc. of 2% slightly acid agar solution. This concentrate was always used at three dilutions equivalent to 10, 20 and 40 cc. serum, and occasionally an 80 cc. concentration was included. Unfortunately, however, curvature of the oats and hormone concentration were not found to be proportional except for very low curvatures. The curvature at the 10 cc. concentration was therefore used to estimate hormone content. The use of three concentrations was retained as a check on the procedure. This range of concentrations was usually sufficient to obtain maximum bending as shown by the form of the graph, but the height of the maximum often appeared to vary with the source of the serum and it was never as great as the indole acetic acid maximum. It cannot therefore be assumed that we are dealing with a single hormone. An example of the difference in the nature of the curves is afforded by those given for slaughter tapped and normally tapped seedling rubber (Graph 2), where curvature in the Avena test against hormone in different quantities of serum is shown.

GRAPH 2.

CURVATURE AND QUANTITY OF SERUM.



In this experiment a few trees in an old seedling area on A.D. tapping were severely slaughter tapped daily for a week before the first sample was taken and a further sample was taken after another week. The graph is the average of the two determinations which were not very different. It will be observed that the hormone is lower in the latex from the slaughter tapped trees. But the form of the curves suggests that the lower curve can never meet the upper.

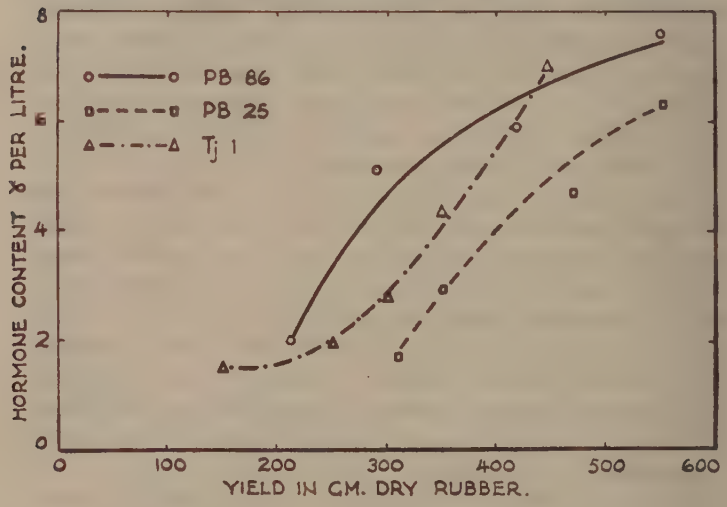
A complementary experiment was undertaken to follow the changes in yield and hormone content of the latex on bringing an area into tapping. The three clones Tj.1, P.B.86 and P.B.25 were selected in adjacent monoclonal blocks. Fifteen trees in each clone were rested for one month and then brought into daily tapping. Initially yield in gm. dry rubber and serum hormone content were highly correlated as can be see from Graph (3). Latex volume was not as closely correlated. After the first four or five days the hormone content of the latex and the yield ceased to be closely correlated. From Graph (4) it will be observed that in each of the three clones the hormone rose to a maximum and then fell sharply before rising to a second maximum. The yield on the other hand remained relatively steady. In Tj.1 it continued to rise for the first three weeks while in the other two clones it declined slowly from the fifth day onwards. Some correlation was, however, shown for the 11 samples taken over 15 days as shown in Graph (5) for the three clones combined. The correlation coefficient is 0.42 with a probability of P = .02.

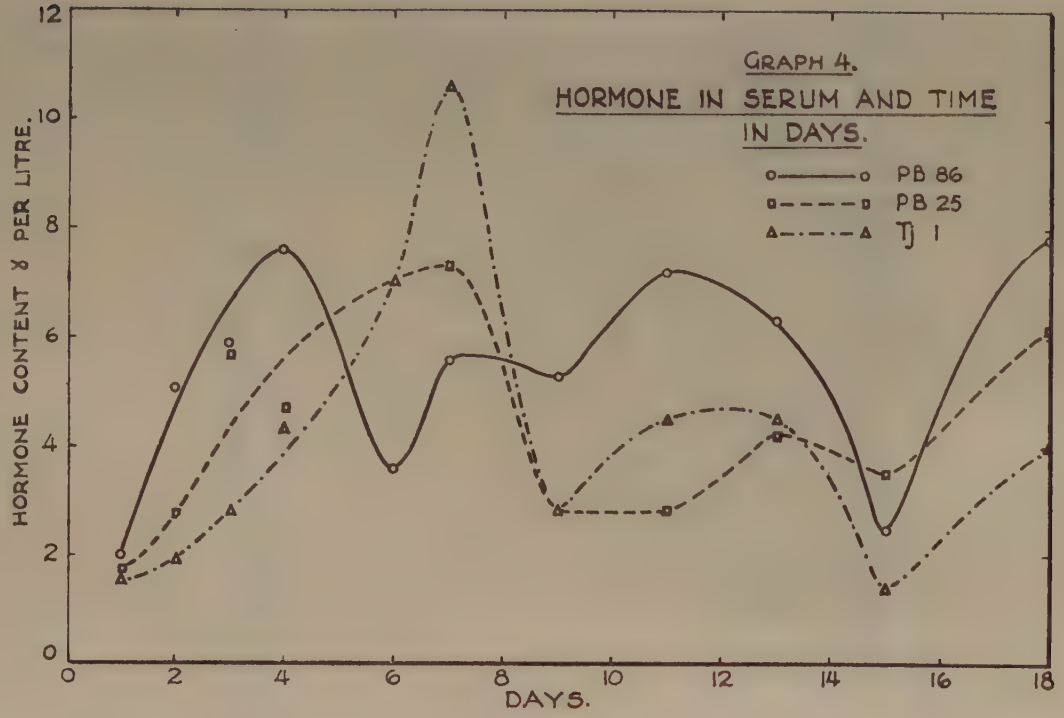
The same three clonal blocks under normal estate half spiral A.D. tapping were also examined for the hormone content of the latex. In Graph (6) curvature in degrees in the Avena test is shown against quantity of serum from which the concentrate was derived. The curves are for averages of five determinations for P.B.86 and three each for the other two clones.

It is again apparent that more than one hormone capable of estimation by the Avena test is probably present. The curves for P.B.86 and Tj.1 are similar, both pass through the same maximum, although Tj.1 contains rather less hormone than P.B.86. But the P.B.25 curve resembles the curve given by the slaughter tapped seedling rubber Graph (2), with its low maximum.

GRAPH 3.

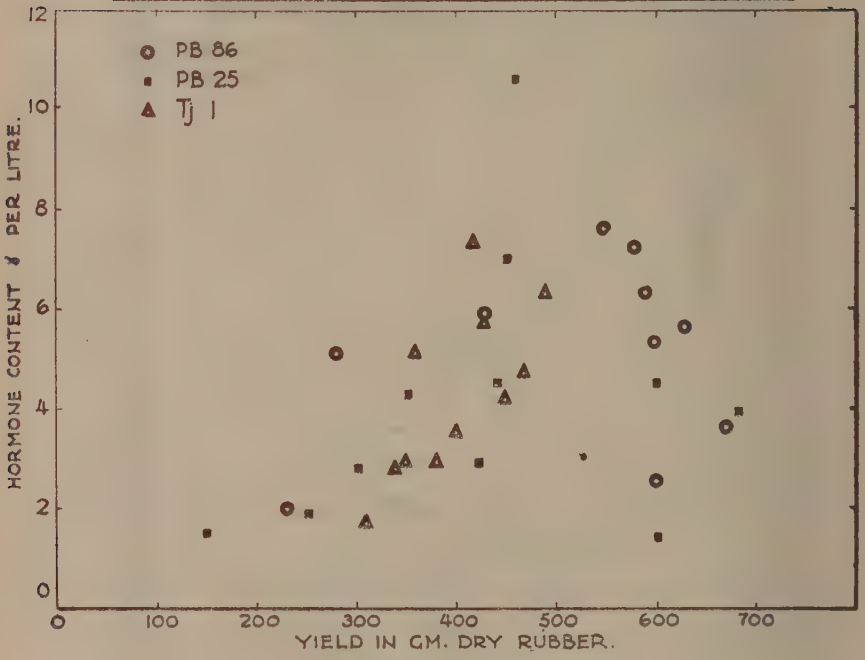
HORMONE IN SERUM AND YIELD IN GM. DRY RUBBER.

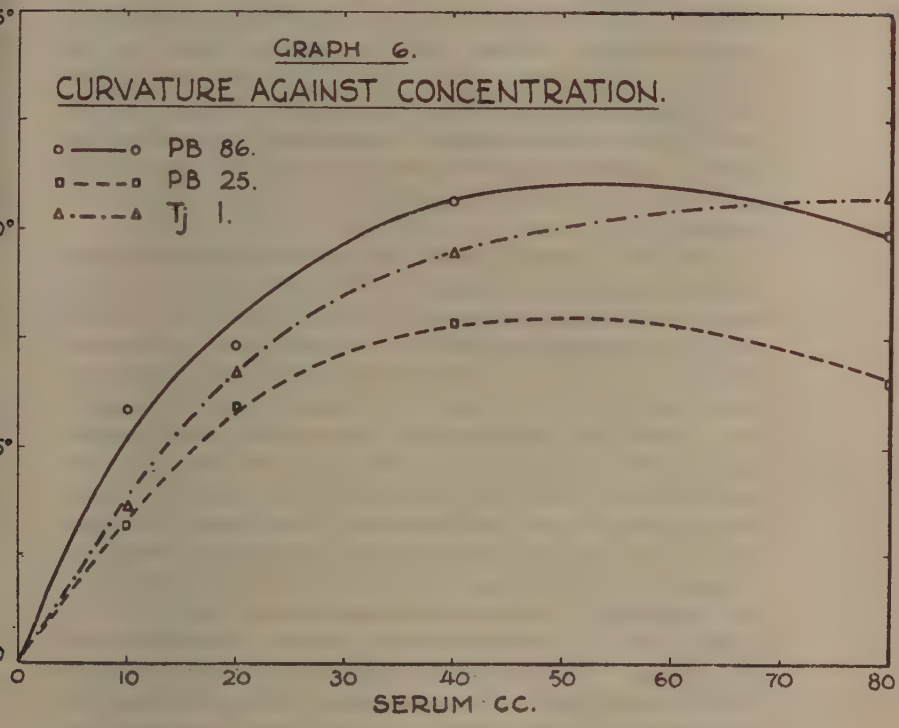



GRAPH 4.
HORMONE IN SERUM AND TIME IN DAYS.
PB 86
PB 25
Tj 1
HORMONE CONTENT % PER LITRE.
DAYS.
0
2
4
6
8
10
12
14
16
18

GRAPH 5.

HORMONE IN SERUM AND YIELD IN GM. DRY RUBBER.




GRAPH 6.
CURVATURE AGAINST CONCENTRATION.
PB 86.
PB 25.
Tj 1.
0
10
20
30
40
50
60
70
80
SERUM CC.

Since high and low yielders react very differently to hormone treatment a series of determinations was carried out on the hormone content of high and low yielders in different areas. On estate 'C' the high yielders contained more hormone.

Seedlings	7.0 γ per litre	4.6 γ per litre
P.B.86	6.8 „	5.7 „

But on estate 'B' in an old area which was being tapped very drastically, though not actually slaughter tapped the high yielders had less hormone than the low yielders. The form of the curve for the high yielders was the same as that for the slaughter tapped rubber, Graph (2). It is therefore suggested that under very heavy tapping the highest yielding trees are most affected and their latex hormone content falls to the slaughter tapping level.

As zinc deficiency in Citrus and a few other species produces deformities of shoots and leaves similar to those which can be produced by 2:4-D a few trees were injected with zinc sulphate crystals by drilling a hole in the base of a large branch. A reduction in yield resulted but no tree was dried up as recently reported by Compagnon and Tixier (5). This may be attributed to the fact that only 2 gm. zinc sulphate were injected far from the tapping panel to avoid damage to the tree. Following the report by the same authors that copper increased yield it was decided to test the effect of copper on latex hormone. Three groups of P.B.86 trees were used. One was untreated, the second was only scraped and the third scraped and 5% copper sulphate applied. When the effect of scraping had worn off as judged by a similar yield in both scraped and unscraped trees the hormone was determined. It was found to be the same in the scraped and unscraped but was higher in the copper treatment at 6.1 γ per litre against 4.9. The yield was also 20% higher for the copper treatment.

Conclusions

Considering the above results it must be remembered that the water phase in latex is drawn from several different types of tissue in the process of tapping. The hormone found in the latex may therefore not be evenly distributed amongst these tissues in which case the

analytical results may not present a true picture of the hormone concentrations in the wood and bark of the tree. There is, however, fair evidence that hormone in latex is influenced by tapping which can either increase or decrease the hormone according to its intensity. The curves further suggest that even the nature of the hormone is not constant. Under these conditions it is not surprising to find that the hormone content of latex is often not correlated with yield although the application of artificial hormones to the bark increases yield.

The great difference in response to hormone treatment of the same clone in different areas is remarkable as is also the difference in response to simple bark scraping. Normally the latter produces only a slight temporary rise in yield as is well known amongst planters, but other Malayan work (*loc. cit.*) affords an example of a large increase obtained by this method.

Yield can be increased not only by scraping the bark but also by fire damage, arsenical poisoning and injecting copper—Compagnon & Tixier (*loc. cit.*). It would therefore appear that natural hormone is produced in response to tapping and various other forms of wounding. If this is correct the differences in behaviour between the areas mentioned above should be explainable from a better knowledge of the hormone metabolism of the tree.

The reduction in yield by the application of hormones above the tapping cut is curious and is difficult to explain on the hypothesis that they produce more latex. If however they bring about a local change in the water relations of the tissues either by altering permeability or osmotic pressure an explanation might be possible. The higher bark content of divalent metals in high yielders may be of significance in this connection.

The conclusion may reasonably be drawn that the artificial hormones are acting in a similar manner to the natural ones and may be described as supplying a natural deficiency from the point of view of yield.

Acknowledgments

The writer wishes to express his thanks to Messrs. Guthrie & Co. for permission to read this paper and for the co-operation of the Managers of the estates on which the field work was carried out.

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(2) Rubber Res. Scheme (Ceylon) Ann. Rept. 1942, 1944 and 1945.

(3) JENSEN B.P., 1936, Translation, New York, *Growth Hormones in Plants.*

(4) WENT F.W. and THIMANN K.V., 1937, New York, *Phytohormones.*

(5) COMPAGNON P and TIXIER P., 1950 *Rev. Gen. Caout.*, **27** Nos. 9, 10, 11.

February, 1951.

Paper No. 3.

Preliminary Studies on the Physiology of Latex Experimental Work by E. G. B. Gooding

(Presented by C. C. T. Sharp and C. W. Brookson)

(Rubber Research Institute of Malaya)

The following account of experimental work carried out by Mr. E. G. B. Gooding has been prepared by Mr. C. C. T. Sharp and Mr. C. W. Brookson from Mr. Gooding's reports. A complete account will be published at a later date.

In this account only qualitative results are given. The quantitative data will appear in the complete papers.

It has been shown by previous investigators that the latex in the latex vessels becomes diluted as the result of tapping. This process of dilution can for convenience be separated into two parts; the first the slow and steady dilution that occurs when a tree is tapped for the first time or after a long rest which may last for several weeks until a constant concentration is reached; and the second the dilution that occurs at each tapping which is followed by a reconcentration within a few hours.

The preliminary investigations described here are designed to throw light on the extent of the area affected by the dilution and to measure the time required for the effect of tapping on the concentration of total solids in the latex to reach different parts of the tree.

It has been found previously by Pyke who made measurements with a dendrometer of his own design that trees in Malaya undergo a daily fluctuation in girth which may reach as much as 0.25 mm. The maximum girth is reached during the night and no contraction can be observed before 7.30 a.m. (Malayan Time). In leafless trees the contraction is very small.

Dendrometers were placed one inch below the tapping cut on trees in full foliage and on wintering (defoliated) trees. The trees were tapped at 6.30 a.m. and the contraction on tapping was found to be swift and marked in both sets of trees. Recovery set in soon after tapping. In trees with a normal foliage this recovery was masked by transpiration effects which occur between 7.30 a.m. and 8.00 a.m. In defoliated trees, where the daily contraction is no more than 5-6 μ, this effect is not masked.

For this reason, defoliated trees were used for the next experiment. Dendrometers were placed on defoliated trees, one inch below the tapping cut, one foot below the cut, and two feet below the cut. After tapping at 6.30 a.m., the contraction one inch below the cut (A) was immediate (Fig. 1). At one foot below the cut (B) the contraction appeared 4 to 6 minutes later, and at two feet below the cut (C), at 8 to 10 minutes after the cut had been opened. With progressively increasing distance from the cut, the contraction was smaller, and the recovery appeared to be slower. Pyke has previously shown that no perceptible contraction occurs above the tapping cut.

It is believed that this contraction of the latex vessels is the result of their partial collapse under pressure from the surrounding tissue and that this partial collapse would be followed by a dilution of the latex by the fluid contents of the cells surrounding the latex vessels passing from the area of high pressure to the area of low pressure in the latex vessels. No method has yet been devised of measuring the internal pressure in a latex vessel but if dilution is the result of a drop in the internal pressure, a measure of the dilution should give an indication of the extent of the drop in pressure.

In the next part of the investigation Ferrand's Latex Drop Micromethod was employed. This method does not differ in essentials from that used by Frey-Wyssling and other earlier workers and need not be described here in detail. It is believed that the first few drops of latex which exude from an incision have been forced out by the turgor of the vessels and the surrounding cells and, having undergone no dilution, will be representative of the latex in the vessels. It is probable that material from the sieve tubes and other cells will be forced out on cutting. Although the contents of the parenchyma cells may be very small, the contents of the cut sieve tubes may be considerable. No experimental check of this could be made and the possibility of contamination of the samples had to be accepted. A sample of successive drops from the same incision however showed no appreciable difference between the percentage total solids of the first and the sixth drop, after which some dilution became apparent. It appears that some latitude in sampling is permissible.

Experiment I. A single tree of clone Glenshiel 1 (girth 24 inches at 30 inches from the ground) was

DENDROMETER EXPERIMENT

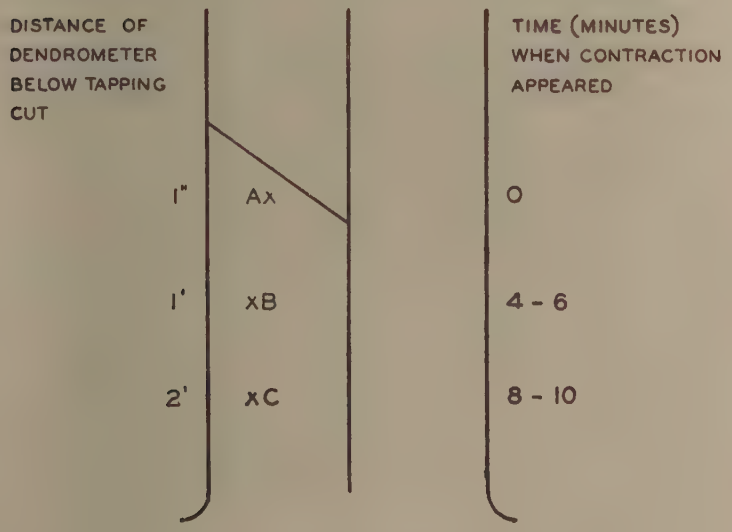

FIG. 1

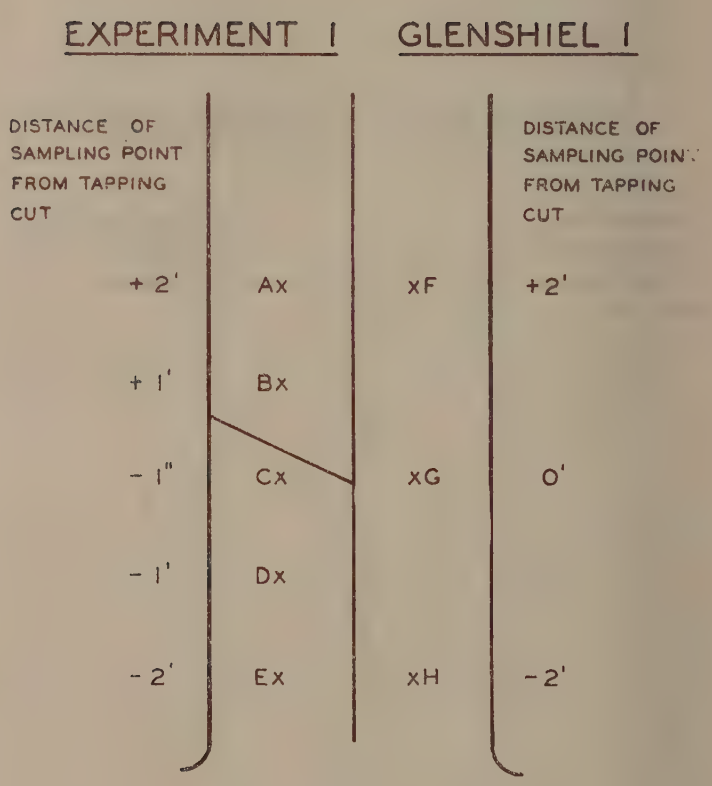
EXPERIMENT 1 GLENSHIEL 1
DISTANCE OF SAMPLING POINT FROM TAPPING CUT
+ 2'
+ 1'
- 1"
- 1'
- 2'
Ax
Bx
Cx
Dx
Ex
xF
xG
xH
DISTANCE OF SAMPLING POINT FROM TAPPING CUT
+2'
0'
-2'

FIG. 2

sampled immediately before tapping on a half spiral cut, at about 8.00 a.m., and again two hours after the cut had been opened, but when the latex flow had ceased. The samples were taken (A) at two feet above the tapping cut, (B) at one foot above the cut (just above the top of the renewing bark), (C) at just below the cut, (D) at one foot below the cut, and (E) at two feet below the cut. On the other side of the tree samples were taken at a height of (F) two feet above the level of the tapping cut, (G) at the level of the tapping cut and (H) at two feet below the level of the cut. (Fig. 2).

At two hours after tapping no dilution occurred at A, B and F, but considerable dilution was found at C, D and E below the tapping cut and at G and H on the opposite side of the tree.

Experiment II. A second more detailed experiment was carried out on a larger tree (girth 50 inches at 40 inches above the union) of clone Tjirandji 1. Twenty-five sampling points were selected from one foot above the union to six feet above the union, and encircling the trunk. Samples were taken from these points at 7.00 a.m. and 9.30 a.m. on seven occasions before the tree was taken into tapping, to give an estimate of the differences that might occur between different samples at the same spot at different times. The tree was then brought into tapping on the S/2, d/2 system, but no samples were taken for a month to allow the latex flow to reach its maximum. Pairs of samples were then taken from the same positions on seven occasions at the hours of 7.00 a.m. and 9.30 a.m. on tapping and non-tapping days, the tree being tapped on tapping days between the two sampling times. It was found that at all sampling points the latex had been affected by tapping, with the possible exception of the point immediately above the centre of the cut. Apart from this the smallest dilution was found at the highest sampling points, two feet above the level of the cut.

Experiment III. A further experiment was carried out using eighteen 10-year old trees. Nine were rested for ten days before sampling, and the other nine were kept on the S/2, d/2 system. Each group of trees consisted of one tree of clone Tjirandji 1, and four trees each of clones Pilmoor B.84 and R.R.I.501; the mean girth of the trees being 24 inches at tapping height. Samples of latex were taken at one inch below the tapping

cut, and at one foot and two feet below the centre of the cut at intervals of 5, 10, 15, 30, 60, 90 and 120 minutes, and 3, 4, 6, 24 and 48 hours after tapping. Samples were also taken at the level of the tapping cut on the reverse side of the tree at less frequent intervals.

The results for all trees were in fair agreement and the means showed that within five minutes of tapping there is a sharp fall in latex concentration at both one inch and one foot below the cut, and at 10 minutes the concentration is lower still, at both positions. No fall of latex concentration appeared at two feet below the cut at five minutes from sampling, but a sharp fall appeared at 10 minutes. At 15 minutes the latex immediately below, and at one foot below the cut showed a small rise in concentration, but at two feet below the cut there was no appreciable difference. At 30 minutes latex total solids at one inch and at one foot below the cut had declined again, and at two feet a decline was also apparent, the decline in these three positions being maintained until about 1½ hours after tapping. By two hours a considerable rise in latex total solids appears at one inch and one foot below the cut, no rise appearing two feet below the cut until three hours after tapping. Latex flow ceased in all trees between two and two and a half hours after tapping. Throughout most of the tapping flow period the latex concentration at two feet below the tapping cut is rather higher than at one foot. On the reverse side of the tree at the level of the tapping cut the changes were smaller and slower to appear. The drop in concentration occurred 30 minutes after tapping, the minimum at three hours, and reconcentration at about six hours. These results are shown diagrammatically in Fig. 3.

Experiment IV. Finally an experiment was undertaken to determine to what distance in the tree the drop in concentration initiated by the tapping cut might extend. A tree of clone Ct.88 (girth 54 inches) which had been resting for several years was selected. Samples were taken at 3 ft., 10 ft., 20 ft. and 33 ft. above the ground; six sets of samples being taken before tapping commenced. The tree was then opened at 36 inches from the union on the S/1, d/4 system and samples were taken daily for 40 days. On the ten tapping days in this period the sampling was done before tapping. Latex total solids below the cut started to decline after the second tapping,

Fig. 3. *Time required for Dilution Reaction to be observed.*

Distance below Tapping Cut of Observation	Time									
	5 mins.	10 mins.	15 mins.	30 mins.	60 mins.	90 mins.	120 mins.	3 hrs.	4 hrs.	6 hrs.
1 inch -	–	–	+	–	0	0	+			
1 ft. -	–	–	+	–	0	0	+			
2 ft. -	0	–	0	–	0	0	0	+		
0″ (reverse side)	0	0	0	–	–	–	–	–	0	+

Latex flow ceased

– = reduction in concentration.

+ = increase in concentration.

0 = no apparent change in concentration.

After the last symbol in each line there was a slow but steady increase in concentration up to 48 hours when the next tapping took place.

shortly followed by those at 10 ft. At 20 ft. the decline appeared after the third tapping and at 33 ft. at about the same time. The final concentration after the tree had been in tapping some time was slightly higher at the upper level than at the lower.

Experiment V. A similar experiment was made on a tree of clone Tjirandji 1 (girth 50 inches) which was opened at 36 inches from the union on the S/2, d/2 system. Samples were taken at heights above ground level of 2 ft., 10 ft., 20 ft. and 26 ft. An effect at 2 ft. was found after the first tapping, and at all other points after the second tapping. From then on the latex concentration at all points fell rapidly from 60% total solids to rather less than 30%.

From the data thus assembled a picture can be built up showing the course of events when a tree is tapped. Tapping causes a contraction of the trunk. This contraction is extremely rapid in the region just below the cut, and the inference can be made that the latex vessels are undergoing a partial collapse under the pressure of the surrounding bark tissues. The collapse spreads only slowly, following the initial reduction of pressure.

It is unnecessary to reproduce here the well-known diagram of Frey-Wyssling in which is shewn the area from which the latex must be extracted at each tapping. Although the general picture of the response to tapping still remains substantially the same it is necessary to modify it in two important respects.

In the first place the experiment with Glenshiel 1 shows that the area from which latex is extracted covers the whole circumference of the tree up to the level of the tapping cut and the experiment with Tjirandji 1 shows that it may even affect the whole circumference of the tree to a height of 2 feet above the tapping cut except possibly for a small area immediately above the centre of the tapping cut.

The second important point is the effect of continuous tapping on the concentration of latex at points many feet above the tapping cut. In Experiments IV and V it was shown that when trees of Ct.88 and Tj.1 were tapped for the first time or after a long period of rest a reduction in total solids in the latex could be demonstrated at a point 33 feet from the ground after the 2nd or 3rd tapping

and that the concentration eventually fell almost to the level of that of the latex immediately below the tapping cut.

It has been observed that when a rubber tree is taken out of tapping the total solids in the latex increase until they reach a certain maximum. It must surely follow that if the concentration of the latex in the branches of a tree falls to 30% from 60% that latex is being manufactured in the branches and that the whole tree is contributing to the crop harvested.

The experiment with the eighteen ten-year-old trees confirms that the effects of tapping are transmitted through the latex system relatively slowly, and the resemblance to the results obtained with the dendrometer will be noted. That greater resistance is met by latex travelling in the horizontal direction is indicated by the observation that the dilution effect took between 15 and 30 minutes to cover the relatively short distance between the cut and the reverse side of the trunk.

February, 1951.

Paper No. 4.

A Study of the Redox System of *Hevea Brasiliensis* Latex

by

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(Institut des Reserches Sur le Caoutchouc en Indochine)

Introduction

It appeared to us that a study of the Redox system of *Hevea Brasiliensis* latex, in addition to its theoretical importance, should afford useful information on rubber metabolism.

Most scientists who have studied the subject have concerned themselves chiefly with oxidation enzymes and these are now well known. Few have considered oxidation and reduction phenomena as a whole.

Van Dalfsen (1) showed in 1937 that latex decolourises methylene blue and the study was recently resumed by Mrs. L. N. S. de Haan-Homans (2). This development was the starting point of our experiments with methylene blue as a redox indicator, and its decolourisation as an approximate measure of the level of the redox potential of the system under observation.

It appeared worthwhile investigating whether the potential is the same for different latices, how it changes with time, and what substances affect it.

Experimental

I. Apparatus

We used a "Heito" pH meter which measures potential differences down to 0.8 m.v., and Beckman electrodes, calomel with saturated potassium chloride and platinum.

The platinum electrode is the positive pole and calculations are as follows:—

The electromotive force measured by the apparatus between the two electrodes is

$$E \text{ (measured)} = 0.0001983\ T\ (7 - L)$$

where

T is the absolute temperature to which the apparatus is set.

L is the reading on the pH scale of the meter.

At 30°C. the potential of the calomel electrodes (with saturated KCl) is 0.242 volt and between 25°C. and 30°C. the variation is very small (0.245 to 0.242 volt).

The redox potential of the system is calculated from the following:—

E red. ox. = 0.242 + E measured.

with reference to normal hydrogen-electrode.

Under these conditions it is possible to measure potential differences from + 0.630 to – 0.170 volt approximately.

Experimental Conditions

Our experiments on methylene blue decolourisation by latex have shown that the phenomena are the same in an atmosphere of nitrogen, with or without presence of air. The following operating technique was used:—

50 grms. of latex are weighed into a 100 ml. beaker, the electrodes are immersed to a depth of 1.5 cm. (they must be carefully cleaned and their zero potential checked before each experiment), and the reading made after a few minutes. The latex should be agitated and its temperature and pH recorded.

All experiments have been made at 25°C. to 30°C. and pH 6 to pH 7. Under these conditions the precision of our readings is about ± 5 millivolts.

All our results on redox potential are expressed with reference to the normal hydrogen-electrode as the rH notation is not convenient for this work.

II. Study of the Decolourisation of Methylene Blue in Latex

The pH of fresh latex does not change very much during the first hours after tapping, only from pH 6.9 to pH 6.2 approximately.

If the normal redox potential curve of methylene blue plotted against pH is examined, it is noticed that decolourisation occurs at the following potentials and pH:—

pH 6—normal potential + 0.047 volt.

pH 7—normal potential + 0.011 volt.

If 0.005% of methylene blue is added to latex collected within half an hour of tapping, the latex remains blue for a period of time which varies for different latices, but is always longer than one hour. If the same amount

of methylene blue is added two or three hours after tapping, the colour is lost within half an hour, and added four to five hours after tapping, decolourisation occurs very rapidly, within five to fifteen minutes.

This may be explained from redox potential measurements as follows (Fig. 1). If we plot the normal redox potential against time, for latex collected half an hour after tapping, and the methylene blue normal potential in terms of latex pH, therefore as a function of time also, we find these two curves cross at time "t" and at this time the conditions are suitable for decolourisation.

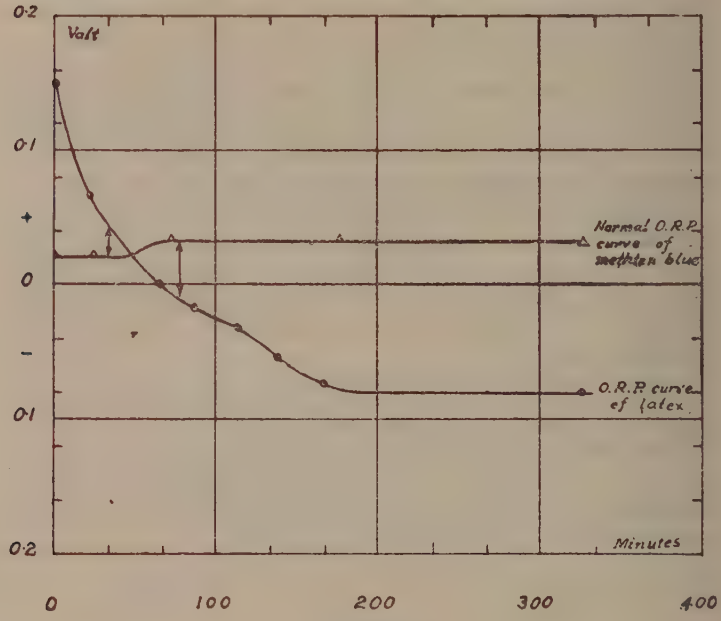

FIG. 1.

It therefore appears that the time necessary for decolourisation depends upon two factors, the redox potential and the latex pH. It is evident however that this conception is theorical since we should consider the oxidation and reduction rates of the different compounds present. The decolourisation begins before the critical point and ends after it, and the time of decolourisation is more directly proportional to the potential differences between the curves for latex and methylene blue for the same period of time.

It is also found by the use of another redox indicator, indigo carmine, that the activation of the latex redox system is towards the negative potential.

Van Dalfsen showed that ammoniated latex decolourises methylene blue, the pH of the latex preserved with normal amounts of ammonia is about pH 11, under these conditions the normal potential of methylene blue becomes equal to – 0.100 volt. Ammoniated latex must therefore have a more negative redox potential to permit decolourisation, in fact we have observed that even with several months old preserved ammoniated latex, there is a redox potential from – 0.120 volt to – 0.140 volt.

Centrifuged latices preserved with ammonia do not decolourise methylene blue, their potential is about + 0.050 volt, on the contrary centrifuged skim latices have a strong negative potential (– 0.170 volt) and decolourise methylene blue very quickly.

Creamed latices generally retain the same redox potential as the latex from which they are made.

III. Study of the Variation of Latex Redox Potential During Tapping

In the previous experiments the latex used was collected half an hour to one hour after tapping and included all latex from the beginning of tapping. It is interesting to follow the latex redox potential from the very first moment of tapping and its subsequent variation.

The experiments were carried out as follows. Twelve trees provided with clean cups were tapped in one and a half minutes. Latex was collected five minutes after the commencement of tapping and the potential measure-measurements made after another five minutes. Subsequently at half hourly intervals until no more latex flowed, samples were collected over a period of five minutes flow. Changes with the time were followed on each sample collected. Additionally, a sample was examined representative of the total quantity of latex obtained during the tapping.

The behaviour of all samples was similar, their redox potentials start at about the same positive level and then changed quickly to negative potentials.

The change is more rapid in the first samples taken. It is easy to understand therefore that latex collected at a certain time after the tapping of the trees (say half an hour or one hour) will have a redox potential higher than the first sample, but lower than the last. The

curve of potential variation of such a latex will be less steep than that for latex from different times (Fig. 2).

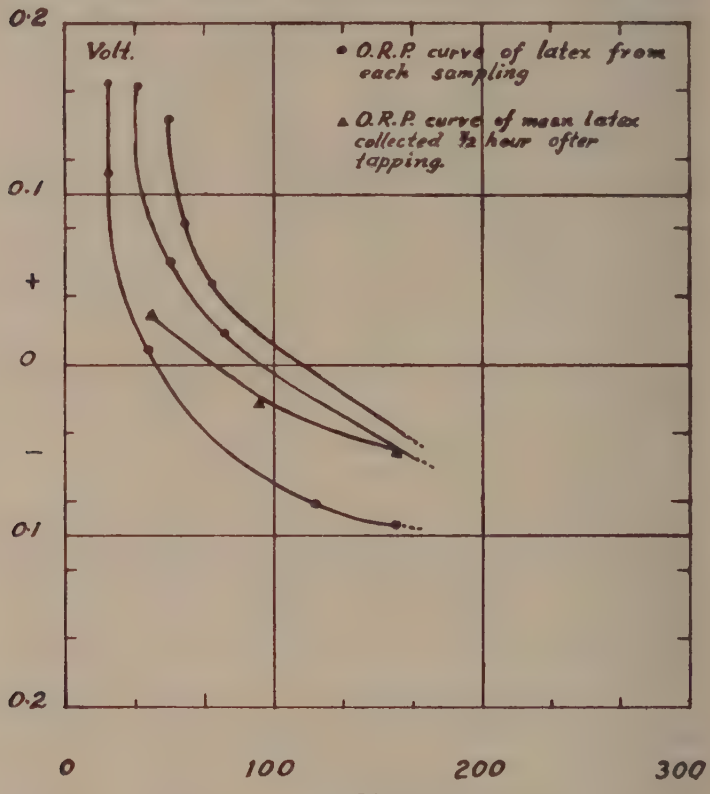

FIG. 2.

We have made also several measurements on the white and yellow fractions of latex described by Van Gils and his co-workers (3), the results were as follows at a given time of collection:—

Total latex	.. ..	– 0.022 volt
White fraction	.. ..	– 0.022 volt
Yellow fraction	.. ..	– 0.010 volt

A slight difference can be noticed, it is however not very significant.

IV. Determination of the Nature of Reducing Compound in Latex

The above experiments prove that there are in latex one or more reducing compounds showing themselves by

these redox potentials. An enzymatic process may be considered as responsible, or the enzymes themselves have reducing groups, or perhaps a reducing compound is produced during the enzymatic reaction which occurs in latex once it has left the tree.

To throw some light on this problem, we have experimented with several enzyme inhibitors. Latex collected from fifteen to thirty minutes after tapping was divided into several fractions, to some inhibitors were added at a given time, and the others kept as controls. All were tested at regular intervals to obtain redox potential curves as a function of time.

The inhibitors of enzymes containing iron and copper (catalase, oxidase, tyrosinase, peroxidase) do not change the general aspect of the redox potential curves. We used potassium cyanide (2×10^{-3} M), sodium azide (7×10^{-3} M), sodium fluoride (5×10^{-3} M), the molecular concentrations being given for one litre of latex.

Sodium oxalate (1.5×10^{-3} M), para-nitrophenol (1×10^{-3} M) are inactive, chloroform (6×10^{-4} M), ammonium sulphide seem to activate the process slightly. Chloroform is known to be an inhibitor of the enzymes belonging to the dehydrogenase group. Ammonium sulphide is a precipitant for heavy metals. Manganese sulphate (1×10^{-3} M) also seems to favour negative potentials.

On the contrary, the salts of heavy metals proved to be very effective in preventing a tendency towards negative potentials, copper sulphate $^{++}$ is effective at a concentration of 1×10^{-4} M, and mercuric chloride $^{++}$ for a concentration of 7×10^{-4} M.

These metals are considered as denaturants of proteins, but also as reactives of – SH groups with which they can build complexes. These results led us to use other reactives of the thiol class as iodoacetic acid and the arsenic salts.

We found a similar behaviour for these compounds, iodoacetic acid or, still better, the alkaline iodocaetates are effective at concentrations about 1×10^{-3} M, the trivalent arsenic chloride at a concentration 5×10^{-4} M.

The different nature of the action of these compounds must be emphasised. The copper salts act almost immediately, but their effect decreases with time, while the effect of iodoacetic acid increases with time (Fig. 3).

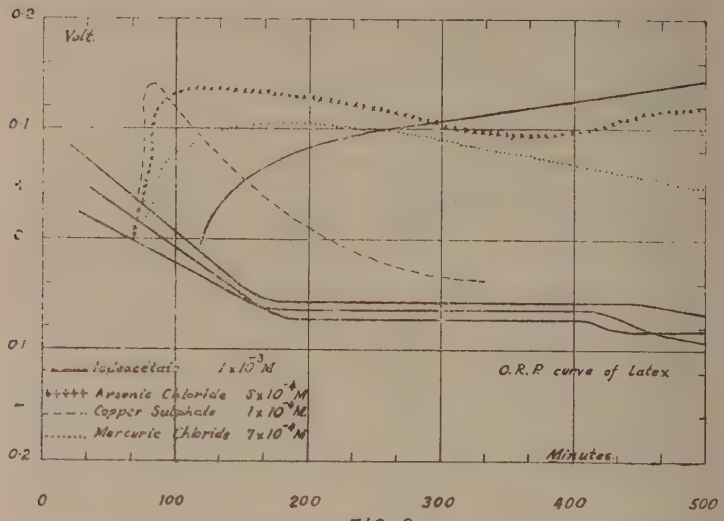

FIG. 3.

This may be due to the destructive action of the iodoacetic acid on the enzymes (irreversible action) whilst the salts of heavy metals have a reversible action.

We may also think that the compound reacting with SH group and producing complexes suppresses the latex negative redox potential. They can act either at the beginning of the process or at the end of it.

A direct consequence of the suppression of the negative redox potential is the abnormal action of oxidase and peroxidase which discolour the latex surface, whilst the control remains white and keeps its negative potential.

From the study of the redox system of latex, the following points emerge:—

(a) The reactives precipitating heavy metals do not modify and sometimes even promote the formation of negative potential in latex.

(b) The reactives of the thiol function suppress the negative potential and bring the redox potential back to the original level present in the first minutes after tapping. This suggests that the negative redox potential which occurs in latex is related to the presence of a thiol function.

V. Study of Latex of Different Vegetative Origins

The study of latex from various vegetative origins was somewhat disappointing, the analogies and differences observed could not be interpreted.

To obtain significant results, a long study is needed and the experimentation would be very difficult, as it will be necessary to observe several kinds of latex on the same day to eliminate most of the variability factors.

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February, 1951.

Paper No. 5.

The Injection of Trace Elements, especially of Copper in the form of Sulphate, into the *Hevea Brasiliensis* Tree

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Introduction

In a publication appearing in *Rev. Gen. Caout.*, **27**, 524, 591, 663 (1950), Compagnon and Tixier have shown that the injection of a few grams of copper sulphate into Hevea brings about an increase in the production of rubber, which seems to last from three to five months and is in the neighbourhood of 40% to 60% over the controls. It is further observed that the rubber content of the latex of the injected trees is less than that of the control latices.

The object of the present communication is to bring forward some new information relative to this increase in production in the light of results from experiments carried out in the course of the year 1950.

These experiments are chiefly relating to copper as sulphate; this salt was the first tried and with it the response was the best. Nevertheless other salts and chemicals have been tried. Information on typical results obtained with some of them will be given.

I. Injecting Copper as Sulphate

A. Experiments carried out since the beginning of the Study

May	1949	..	Experiments on four twin pairs.* (No further re-injecting).
October	1949	..	Experiments on four twin pairs. (No further re-injecting).
December	1949	..	Experiments on three twin pairs. (Re-injected every six months).
January	1950	..	Experiments on budded Djasinga 1; injecting at different heights. (No further re-injected).

* For all the experiments carried out on twin pairs, one member of each pair was injected, the other served as a control.

May 1950 .. Experiments on 20 brown-bast seedlings. (Re-injected after 6 months).

July 1950 .. Experiments on 200 budded Tj.1 trees put into tapping 3 months before injection. (Part of them were re-injected in January, 1951).

August 1950 .. Experiments with Copper acetate instead of Copper sulphate on three twin pairs.

October 1950 .. Experiments with different quantities of Copper sulphate on budded Djasinga 1.

November 1950 .. Experiments on 100 budded AV.50 (1934 planting), 100 of the same budded trees used as a control.

All experiments were made on 1941-1942 plantings except the last.

B. Response Period and Consequences

The response period depends on various factors not yet known. Usually a definite increase in the weight of rubber produced can be observed over a period which exceeds three months and usually lasts for five or six months.

The figures given in Table I relate to the May 1949 experiment and show the production and the D.R.C. after one year.

Table I

Experiment of May 1949

Observations from the 13th to the 18th month after Injecting

	Monthly Yield in gms.		d.r.c. Monthly mean	
	Injected	Control	Injected	Control
June 1950 -	1734.7	1671.2	32.5	35.1
July -	1720	1490.7	30.7	33.3
August -	1823.6	1534.6	32.1	34.5
September -	1497.6	1235.1	30.5	32.9
October -	1670.1	1720.2	28.9	30.9
November -	1865.2	1924.5	33.4	34.6
Mean -	1718.5	1596	31.35	33.55

The figures in Table II give the quarterly production over a period of 15 months for one twin pair of the October 1949 experiment. The response of this twin pair to copper was especially high over a period of nine months.

TABLE II

Quarterly production from a twin pair of the October 1949 Experiments

	Injected Yield in gms.	Control Yield in gms.	Yield % of the control
October 1949 / December „	2342.5	962.2	243%
January 1950 / March „	2148.6	1250.2	171%
April 1950 / June „	3155.4	1811.5	174%
July 1950 / September „	1803.6	2054.8	87%
October 1950 / December „	2448.4	1783.0	137%

From these two tables it appears that the immediate yield increase is not obtained at the expense of future production.

C. RE-INJECTION OF THE TREES

Successive re-injections might be a method of forcing the trees and so indicate adverse effects from the injections.

The trees used in the December 1949 experiments were re-injected for the first time on June 1950 and for the second time on December 1950, i.e. every six months. The production relating to the first month following each injection is given on Table III.

TABLE III

Mean production by tapping day relating to the first month after each injection on the same trees

	Injected gms. / tapping day	Control gms. / tapping day	% of the control
December 1949 / 1st Injection	155	99.7	155%
June 1950 / 2nd Injection	170.1	102.1	166%
December 1950 / 3rd Injection	193.1	104.1	185%

It appears that re-injection is not detrimental to future yields. an increase in production of the treated trees is even observed which is not evident in the controls. No sign of harmful effect appeared on bark or foliage. This experiment is to be continued in the same way.

D. Influence of the Amount Injected

Experiments with various quantities of copper sulphate, from 3 grams to 12 grams per tree gave very similar results. There is no proportionality between the response of the tree and the amount of copper sulphate used. This experiment will show later if the response lasts for a longer time with large amounts.

E. Effect of Copper Acetate Instead of Sulphate

The increase in production was very small and the effect lasts three months. The D.R.C. remained the same. It must be noted that the salt was acid which has perhaps an adverse affect due to low pH.

F. Injecting Brown-Bast Trees

Twenty brown-bast seedlings of 1941 planting (with dry tapping-cuts and brown bark but without trunk distortion) were selected. and each received 4 gms. of copper sulphate at the beginning of June 1950. They were re-injected in December. Production is given in Table IV.

Table IV

Mean production of Brown-Bast trees for each Tapping Day

MONTHS	YIELD gms./tree/ tapping day	Observations
June -	4.36	First injection
July -	10.76	
August -	15.90	
September -	7.41	
October -	7.05	
November -	4.54	
December -	9.11	Re-injection

The following observations are made:—

Injecting copper sulphate appears to be a possible mean of curing brown-bast trees, except for distorted trees.

One only among twenty injected trees remained dry.

The yield of these treated trees was very irregular from one tapping day to another.

The rapidity of response and the period of response are very variable for different trees.

G. Effect on different Planting Materials

Besides the experiments mentioned above, applications have been carried out on several estates and it appears that an increase of the order of 50% cannot be considered as a general rule. Big differences in reaction are observed according to the plant material used, and the same clones have not reacted in the same way in different localities. From the results on one estate where many experiments were carried out, a greater increase (up to 190%) seems to be obtained on old trees. In this connection, we observe no increase at all for our July 1950 experiment on Tj.1 trees, where the trees were in tapping for only three months.

If Copper is involved in the formation of rubber from carbohydrates, the response may be dependant on the disposable amount of carbohydrates, and in certain cases carbohydrates would be the limiting factor, then according to this hypothesis no response is to be expected. Bark analysis could give information on this point.

H. The Best Period for Injections

In Indochina the best period seems to be the beginning of the dry season. Three arguments support this view.

During the wet season a great part of the production increase is included in the afternoon latex collection; with afternoon rains this overproduction is lost.

The flow of latex is normally at a maximum in the first part of the dry season, injecting trees at the beginning of this period gives an increase in production which is not the highest in per cent, but is the maximum in rubber weight.

As said in the paper mentioned above, the recovery in yield after the rest period is more rapid in the injected trees.

As an example, Table V gives recent figures from our November 1950 experiment on Avros 50.

TABLE V

Experiment on Av.50 budded trees (Injected on 10.11.50)

	YIELD gms./tree/tapping day		Percentage of the control
	Injected trees (98 trees)	Control (102 trees)	
From 13.11.50 to 1.1.51 -	119.0	89.0	33.6%
From 1.1.51 to Rest Period (Rest Period: from 2.2.51 to 19.2.51) -	100.5	87.1	15 %
From Rest Period to date (10.3.51) -	42.4	23.2	82.8%

I. TECHNOLOGICAL PROPERTIES OF RUBBER FROM INJECTED TREES

Investigations have been made on a semi-industrial scale, (i.e. July 1950 and November 1950 experiments) on the latex from the injected trees and the properties of rubber therefrom.

Mooney Viscosity and Modulus values which are used in the testing of "Technically Classified Rubbers" were determined. The results are given on Table VI.

We see that the modulus of the rubber from injected trees is significantly increased and the Mooney Viscosity is lower.

According to the paper of Compagnon and Cretin (Paper No. 28 of this Symposium), this modulus increase is correlated with D.R.C. decrease and nitrogen content increase. The D.R.C. decrease of injected trees latices is well known, and a few analyses show a slight increase in the nitrogen content of the latices.

It is worth noting that for the July 1950 experiment on Tj.1 no increase in production was obtained, but nevertheless the latex D.R.C. was lower and the rubber modulus higher than for the control.

TABLE VI

Mooney Viscosity and Modulus at 600% of rubber from Injected trees and controls

			Mean	Student's "t"	Level of significance
MOONEY VISCOSITY	Tj.1 (July Exp.)	Control	74.1	1.636	P = 0.1
		Injected trees	71.5		
	Av.50 (November Exp.)	Control	89	2.495	P = 0.05
		Injected trees	80.7		
MODULUS AT 600%	Tj.1 (July Exp.)	Control	42.1	2.837	P = 0.02
		Injected trees	48.6		
	Av.50 (November Exp.)	Control	26.1	2.642	P = 0.05
		Injected trees	31.7		

It must be also noted that the latex stability in the field appeared better for the injected trees.

II. Injection of Other Elements

All were injected as sulphate except for boron. We shall merely mention here the most important observations.

Boron

Only one experiment was carried out with boric acid. Over twenty weeks the injected trees gave an increase in production of 30% on the control. The effect on the D.R.C. appears very slight.

Manganese

Manganese seems to act as copper, but manganese re-injected into the trees does not give so much response as copper. Moreover the D.R.C. decrease is less with manganese than with copper.

Iron

We observe a D.R.C. decrease as with copper, but only a slight increase in production.

Zinc

It was not confirmed that injecting the zinc salt could stop production, but it appears that zinc has a definite depressive effect on the yield.

2 : 4.D

The effect of this biotic substance has already been discussed (*loc. cit.*). It was seen that injection or application to the bark below the tapping cut gave an increase in the yield and a decrease of the D.R.C. Re-injections experiments with 2:4.D. has now indicated that excess of this product can cause very damaging effects to the bark.

February, 1951.

Paper No. 21.

A Note on the Phosphatase in Latex

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Very little is known concerning the transformations of the phosphorylated constituents of Hevea latex, although isolated observations in these laboratories lead to the view that changes in latex proceed rapidly immediately after it has been tapped. When fresh latex was examined for the presence of a factor which promotes the breakdown of substances containing organically combined phosphorus, it was found to contain a phosphatase which catalysed the hydrolysis of glycerophosphate very strongly between pH 5 and 7.

A systematic investigation was then started which forms the subject-matter of this paper.

Preparation of Latex Phosphatase

Phosphatase-active serum is obtained when fresh latex is coagulated with dilute acetic acid but not when it is coagulated with alcohol. The acid serum is obtained by coagulating 100 ml. of fresh latex with 10 ml. of 5% acetic acid.

Owing to the high phosphorus content of the enzyme preparation present in acid serum, an attempt was made to isolate the phosphatase from other serum constituents, particularly from those containing phosphorus. Fortunately this did not prove to be difficult as the proteins precipitated from acid serum by saturation with ammonium sulphate and redissolved in water were found to contain the whole of the active material, while saturation with magnesium sulphate yielded only a slightly less active product.

Solution of the proteins in water and their reprecipitation three times with ammonium sulphate slightly increased the enzyme activity (in one instance from 14% hydrolysis to 18%), and the phosphorus content of the preparation was reduced by 40%. By dialysis overnight a protein solution was obtained containing very little phosphorus. It was fully active after 2 days in the refrigerator.

Thus: Initial % Hydrolysis = 18.91.
After 2 days, % Hydrolysis = 18.63.

An attempt was made to find out in which portion of the serum proteins precipitated by ammonium sulphate the enzyme was present. This involved further concentration of the enzyme by the method of fractional precipitation of acid serum proteins. From trials made with 0.33, 0.35, 0.40, 0.50, 0.67 up to 1.00 saturation with ammonium sulphate, it was found that serum proteins precipitated by 0.35—0.50 saturation with ammonium sulphate possessed the highest activity. For convenience the serum proteins may be divided into three fractions:—

(1) Precipitated by 0.0—0.35 saturation with ammonium sulphate. Only a small amount of proteins was precipitated. The activity of their aqueous solution was practically nil.

(2) Precipitated by 0.35—0.50 saturation with ammonium sulphate. This middle fraction showed the highest activity indicating the presence of phosphatase in this fraction.

(3) Precipitated by 0.50—1.00 saturation with ammonium sulphate. The amount of proteins precipitated in this fraction was the largest, but the activity of their aqueous solution was only slight.

The phosphatase-containing fraction when preserved with a few drops of toluene or chloroform and stored in the refrigerator retained its activity for a fairly long period of time. Samples of enzyme preparations have been tested and found to be active even after 3 weeks' storage.

Method of Determining Activity of Phosphatase Preparations

The method adopted for determining the activity of the phosphatase preparations was as follows: $6\frac{1}{2}$ ml. sodium citrate buffer were added to $\frac{1}{2}$ ml. serum protein solution and then 5 ml. of 0.5% sodium glycerophosphate (substrate) were added. As control the mixture of citrate buffer and serum protein solution was heated to boiling and cooled before the addition of 0.5% sodium glycerophosphate. Both test and control samples were incubated in a water-bath thermostatically controlled at 37°C. After a period of incubation (usually 2 hours), 3ml. of $2\frac{1}{2}$% trichloracetic acid solution were added and the contents

filtered. The amount of inorganic phosphate was estimated colorimetrically from an aliquot portion of the filtrate. (Kuttner and Cohen J. *Biol. Chem.* 1927, 75, 517 and Barnes *et al. J.I.E.C. Anal.* 1944, **16**, 9).

The difference in the amount of inorganic phosphate liberated in the test and control samples gives the amount of organic phosphate which has undergone hydrolysis and this is taken to be a measure of the activity of the serum protein sample.

The activities of protein fractions before, and after, dialysis for 24 hours in a cellophane membrane cell, were compared. Results showed that the activity of the dialysed samples was only slightly higher than that of the undialysed samples e.g.

(1) Serum Protein Fraction (0.35—0.50 saturation with ammonium sulphate)
Before dialysis, % of organic phosphorus hydrolysed .. .. .. = 10.54
After dialysis, % of organic phosphorus hydrolysed .. .. .. = 10.58

(2) Serum Protein Fraction (0.50—1.00 saturation with ammonium sulphate)
Before dialysis, % of organic phosphorus hydrolysed .. .. .. = 5.65
After dialysis, % of organic phosphorus hydrolysed .. .. .. = 5.87

For this reason it was decided to continue using undialysed phosphatase preparations for the present investigation.

Choice of Substrates

In the initial stages, sodium glycerophosphate was used as substrate. In the later stages of investigation, disodium phenyl phosphate (D.P.P.) was preferred as substrate for three reasons:—

(1) D.P.P. gives a higher rate of hydrolysis e.g. with 0.5% sodium glycerophosphate, % Hydrolysis = 20.5, whereas with 0.5% D.P.P. % Hydrolysis = 28.7;

(2) D.P.P. contains a much smaller proportion of inorganic phosphate than sodium glycerophosphate;

(3) With D.P.P. the progress of hydrolysis could be readily followed by determining either inorganic phosphate or phenol (Folin and Ciocalteu J. Biol. Chem. 1927, 73, 627).

Effect of Drying and of Various Substances on Phosphatase Activity

It was found that the active serum protein fraction totally lost its activity after being dried at room temperature in a vacuum desiccator. Thus:—

(a) *Before* drying, % hydrolysis = 16.8

(b) *After* drying, % hydrolysis = 0.0

Ammonia-preserved latex and serum prepared from 2 weeks-old frozen latex show no phosphatase activity. Fresh latex preserved with 0.1% formaldehyde, 2.0% sodium benzoate or full saturation with sucrose has been found to retain its phosphatase activity, though in all these cases as well as in the case of acid serum, the activity diminishes with the period of storage. Ammonia causes an immediate drop in phosphatase activity which is not restored by the addition of magnesium ions.

The activity of fresh latex and of serum protein fraction was partially inhibited by Zn^{++}, Cd^{++}, Cu^{++}, Pb^{++} and CN^{-} ions see Table.

Table

Effect of Inhibitors on the activity of fresh latex and serum protein fraction using 5ml. of 0.5% sodium glycerophosphate as substrate. Period of Incubation 2 hours. Temperature of Incubation 37°C.

Inhibiting Ions	% Inhibition	
	Using fresh latex (2 ml.)	Using serum protein fraction ($\frac{1}{2}$ ml.)
Zn^{++}	38	20
Cd^{++}	39	39
Cu^{++}	37	31
Pb^{++}	20	39
CN^{-}	19	3

In this study of the effect of inhibitors, the concentration of each was adjusted to 0.01M on the reaction mixture. Iodoacetamide was not an inhibitor thus indicating the absence of reactive—SH groups in the system.

Effect of pH on Phosphatase Activity and Choice of Buffers

The effect of pH on the phosphatase activity of serum proteins using disodium phenyl phosphate as substrate was next studied. Two different buffers, acetate and citrate buffers, were compared. With acetate buffer, the optimal pH lies between 5.2 and 5.9. With citrate buffer it lies between 5.7 and 6.4.

Different buffer concentrations were tried and as was to be expected it was observed that the more concentrated buffer had a better buffering effect on the incubation mixture. Thus 0.2M buffer is to be preferred to 0.1M.

The Composition of the buffers was therefore:

(1) Acetate 0.2M acetic acid/0.2M sodium hydroxide.

(2) Citrate 0.2M Sodium citrate/0.2M sodium hydroxide.

The final concentration of the buffer in the incubation mixture was adjusted to 0.1M.

Results showed that although the acetate buffer gave a slightly higher percentage hydrolysis (in one instance 22.4% hydrolysis compared with 21.1%), its buffering action was less than that of citrate. For this reason the use of citrate buffer is to be preferred.

Hydrolysis of Glycerophosphate by Latex Phosphatase

It was found that the enzyme-promoted hydrolysis of glycerophosphate using buffered latex proceeded at a uniform rate during the first 40 minutes of incubation at 37°C (up to 8% hydrolysis) but that thereafter the rate fell. An experiment to test whether the products of hydrolysis inactivate the enzyme showed that glycerol (up to 50% v/v of the incubation mixture) had no effect on the reaction but that phosphate (Na_2HPO_4) had a marked inhibiting effect. This would account for the falling reaction rate on prolonged hydrolysis, and also, since the glycerophosphate substrate used in the experiment contained 8% of its phosphorus in inorganic form, the observation that high glycerophosphate concentration reduces the rate of hydrolysis. The observed result with glycerol and inorganic phosphate is in agreement with the findings of several workers (see H. D. Kay *Biochem. Journal* **22**, 1928, p.855 and M. Maitland and R. Robinson *Biochem. Journal* **21**, 1927, p.665). Increasing the enzyme

concentration, as is to be expected, produced a greater liberation of net inorganic phosphate.

Synthetic Activity of Latex Phosphatase

To test whether latex phosphatase has synthetic activity, two mixtures of buffered latex pH 6.3 (one unheated and the other heated to inactivate the enzyme) with glycerol (50% $^{v}/v$) and inorganic phosphate (Na_2HPo_4, 54 mgm. P per litre of incubation mixture) were incubated at 37°C. After 40 hours there was a drop in the free inorganic phosphorus equivalent to about 20% of the added phosphate. Owing to the high concentration of phosphate in the latex itself, relative to the amount of Na_2 HPo_4 added, this result is not of quantitative significance, but is an indication that latex phosphatase catalyses the synthetic as well as the hydrolytic reaction.

Further Work on Latex Phosphatase

The phosphatase activities of serum proteins and of beef kidney extract were next studied, using disodium phenyl phosphate (D.P.P.) and latex lipoid material as substrates. Latex lipoid material was obtained by the method of Rhodes and Bishop (J. Rub. Res. Inst. Malaya 1930, 2 125). Both enzyme preparations were active towards D.P.P. but not towards lipoid material. Serum protein fractions were shown to have no esterase activity towards ethyl acetate or lipase activity towards coconut oil. It would seem therefore that latex phosphatase plays no part in the breakdown of latex lipoid constituents.

Acknowledgments

Thanks are due to Mr. M. W. Philpott, Head of the Chemical Division, for initiating the research and for extremely helpful criticism and guidance throughout the whole course of the investigation, and to Mr. G. W. Drake for valuable assistance especially on analytical procedures.

February, 1951.

Paper No. 25.

A Chemical Stability Test for Latex

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For practical purposes technologists have come to accept a measurement of the behaviour of latex in the presence of zinc oxide as an index of chemical stability. Several tests have been devised which may be classified as follows:—

(1) Measurement of the change in viscosity with time in the presence of zinc oxide.
(2) Measurement of the change of mechanical stability in the presence of zinc oxide.
(3) Measurement of the change in heat sensitivity in the presence of zinc oxide.

The first is typified by the ZOT (zinc oxide thickening test) described by Murphy (*Proc. Rub. Tech. Conf.* 1938, 151) and the second in the test described by Davey and Coker (*Trans. I.R.I.* 13, 1938, 368). No references to the third method have been found although it is known not to be new. The object of this note is to describe a practical test based on this principle which measures the critical gelling temperature of a latex/zinc oxide mixture under standard conditions as an index of stability.

Apparatus

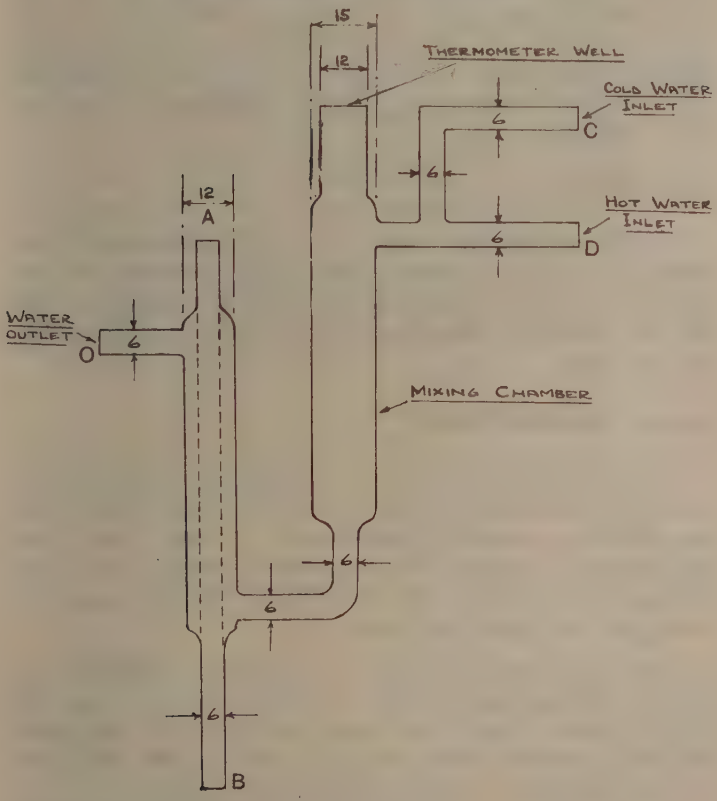
This consists essentially of a glass condenser and mixing chamber of standard size and design (Fig.). The open ended tube AB holds the sample and hot and cold water are fed in at C and D from constant level devices, through needle valves, the water overflowing at O. The hot water supply is from a thermostatically controlled bulk kept at just over 90°C. Standard wall pyrex tubing is used and the dimensions are as shewn.

Method

A standard zinc dispersion is prepared by ball milling together for 16 hours.

Zinc Oxide C.P.	.. ..	50 parts
Water	.. .. ..	47.5 parts
Dispersing Agent (Darvan)	..	2.5 parts

HEAT STABILITY APPARATUS



Standard Wall Pyrex Tubing used.
Drawn full size. All dimensions in M.M.

To a weighed sample of latex of known D.R.C. is added drop by drop on the balance sufficient zinc oxide dispersion to give a proportion of 1% of zinc oxide on the dry rubber. The latex is constantly stirred during the addition of the compounded latex.

Samples are tested 1 hour and 24 hours after the addition of the zinc oxide.

The test is carried out by adjusting the apparatus to 90° then drawing a sample of the latex up into the condenser tube and holding it there for 60 secs. If the latex does not coagulate the zinc oxide stability is reported as $> 90°C$. If it does the test is repeated until the highest temperature is found at which the compounded latex does not coagulate. This temperature is reported as the stability.

Reproducibility

In a number of tests to check reproducibility of results it was found that results agreed within 1°C for different samples of latex drawn from the same bulk lot and for different dispersions made from the same bulk of zinc oxide.

Factors Affecting Tests

(1) Ammonia Content. A series of tests were made using old deammoniated latex to which additional ammonia was added in varying proportions. It was found that the stability increased with ammonia content until the latter reached 0.25 N but that above this concentration there was no significant effect.

(2) Zinc Oxide content. The stability falls rapidly with increasing amounts of zinc oxide up to 1% on the rubber phase. Above this level there is much less effect and it is concluded that 1% zinc oxide is the minimum quantity necessary to give satisfactory results.

(3) Time of Dwell. It has been found by experiment that 60 seconds is the minimum time necessary to establish thermal equilibrium between the latex and the jacket in this apparatus. If a shorter standard time is taken variations of a few seconds make appreciable differences in the result. This is not the case with a 60 second time of dwell. In this connection a fairly vigorous

rate of water flow is recommended to ensure good heat transmission.

(4) Standing Time. This is the time allowed to elapse between compounding and testing. As a result of numerous tests it is recommended that tests after 1 hour and 24 hours are satisfactory for practical purposes.

(5) Dilution of latex with distilled water increases the stability as determined by this test.

Value of Test. It is not proposed here to discuss the theory of the effect of zinc oxide in latex. Unfortunately no data are available to correlate results by this test with other methods of measuring zinc oxide stability. It has however been found that this method provides a quick sensitive and reproducible test for estimating chemical stability. It has the advantage that it can be carried out with cheap and very simple apparatus and is thus suitable for use in the producing territories where sensitive instruments like pH meters are difficult to keep in good condition or to repair. As an example of its sensitivity typical results on commercial samples of bulk centrifuged concentrate are shewn in the appended table. On a latex which had suffered prolonged storage results as low as 52° C were obtained.

Normal Latex—Typical Results

	ZnO No. (Temp. °C)		
Lot No.	1 hr.	4 hrs.	24 hrs.
A	> 90	> 90	> 90
B	80	80	80
C	83	82	80
D	80	80	78
E	86	85	80
F	> 90	> 90	> 90
G	> 90	> 90	> 90
H	80	79	79
I	> 90	> 90	> 90
J	> 90	> 90	> 90

Concentrated Latex—Typical Results

Lot No.	ZnO No. (Temp. °C) 1 hr.	4 hrs.	24 hrs.
A	70	67	66
B	72	71	71
C	80	75	75
D	81	80	80
E	76	76	76
F	90	90	90
G	86	85	85
H	90	90	90
I	90	90	90
J	85	85	84
K	88	88	87
L	79	78	78
M	90	88	86
N	88	88	88
O	90	90	90
P	90	88	88

The practical value of this test to those engaged in the preparation and sale of vulcanised or compounded latex will be apparent.

In using this test as a routine there appears to be some correlation with KOH No. but this is by no means perfect and cases have been found in which two lots of latex of about the same age and almost identical KOH No. differ by as much as 10° in zinc oxide stability. These results are not conclusive as they cover only a limited range since most of the commercial samples handled are good latex recently prepared and fall in the range 88°C to 90°C.

Summary

A simple and useful practical test is described which affords a measure of latex stability in the presence of zinc oxide. Much work remains to be done to correlate this test with other methods of measuring chemical stability and to define exactly what inherent properties of, or constituents in, the latex are being measured. The reasons for its description here are firstly that it does not appear to be generally known and no literature reference has been found; and secondly that it is so quick, simple and sensitive that it is hoped investigators engaged in fundamental work may find it useful, or be induced to provide the correlations sought between this test and more complex analytical procedures.

Acknowledgment

The specification of the test as described here and the data supporting the above note have been provided by P. A. Coffin, E. A. Martin, and their associates of the General Latex and Chemical Corporation, and were largely embodied in a report by these authors to Sub-Committee VII of Committee D-11 of the ASTM dated March 2nd 1948. In this report the authors state "It is believed that this was originally developed by the Firestone Tire and Rubber Co., no literature reference has been found."

Discussion

Dr. Compagnon stated that he had used a somewhat similar method, determining the time required for a latex zinc oxide to gel when held at 70°C and questioned whether this was not a preferable procedure. Dr. Van Gils raised the question of protein coagulation and pointed out that latex may coagulate at or below 90°C without zinc oxide. It was explained that a control heat stability test was always done using the same apparatus and latex, but without zinc oxide. In dealing with fresh concentrates no example of coagulation in the absence of zinc oxide had been found.

Dr. Paton expressed the view that the short exposure at high temperature may be preferable to Dr. Compagnon's method as there was much less chance of complicating side reactions.

February, 1951.

Paper No. 28.

Correlations of some Constituents of Latex and Technical Properties of the Rubber

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Introduction

This paper reports preliminary findings relating to correlations which may exist between the following properties of latex, and between these properties and the nitrogen content and physical properties of the rubbers prepared from them.

Total Solids Content.
Dry Rubber Content.
Total Nitrogen Content.
Copper Content.

The experiments were carried out between the end of September and the beginning of December. This period covers the transition from the rainy season to the dry season in Indo-China during which time a progressive increase in the modulus of rubber is generally noted. It is therefore probable that the results we have obtained may not be applicable for all periods of the year and further investigations are now being made.

Experimental

In these experiments, smoked sheets were prepared under standard conditions several times a month from clonal latices obtained from areas at our experimental station at Laikhe. The fields and clones used were as follows:—

Field V. (1934 clearing) containing clones B.D.5, Tj.1, Av.50, Av.49, O.E.1, P.B.186, Gl.1, Dj.1, S.B.24.
Fields 3/5 and 4/5. (1942 clearing) contaning clones Tj.1, Tj.16, Av.186, Av.188, Gl.1, Av.255, P.R.107, Av.308.

A definite difference is noted between the latex of these two fields. The older field V generally gives a high D.R.C. and a rubber of low modulus, whilst the younger fields 3/5 and 4/5 give lower D.R.C. latex and higher modulus rubber. However there is a wide range

of values for the clones in each field and the upper limits frequently overlap the lower. It is therefore particularly interesting to find out if correlation coefficients established separately for each field remain valid when all results are considered together as a single population.

In calculating the correlation coefficients for any pair of properties, the paired determinations used in the computations have been obtained by using for each clone the means of the individual results for each tapping over the period of the experiment. From these clonal means the correlation was calculated according to the following formula:—

$$r_{xy} = \frac{\frac{\Sigma xy}{N} - \bar{x} \cdot \bar{y}}{\sqrt{\frac{\Sigma x^2}{N} - \bar{x}^2} \cdot \sqrt{\frac{\Sigma y^2}{N} - \bar{y}^2}}$$

where

x and y are the paired results

$\bar{x}$ and $\bar{y}$ are the means of all the results

N is the number of pairs of results

The significance of the correlation coefficients has been tested by using the Fisher table according to the number of degrees of freedom available.

Discussion of Results

Correlation coefficients between various chemical and physical properties of latex and rubber prepared therefrom are shown in the table.

—	N% T.S.	N% RUBBER	D.R.C.	T.S.	MODULUS 600%	Cu mg. per 100 gm. Latex
N% T.S.	—	—	− 0.54 N.S.	− 0.61 N.S.	+ 0.85 H.S.	+ 0.73 S
N% RUBBER	—	—	—	− 0.29 N.S.	+ 0.42 N.S.	—
D.R.C.	− 0.86 N.S.	N.S.	—	—	− 0.85 H.S.	− 0.82 H.S.
T.S.	− 0.68 N.S.	0 N.S.	—	—	—	− 0.76 S
MODULUS 600%	+ 0.90 H.S.	+ 0.39 N.S.	− 0.88 H.S.	—	—	—
Cu mg per 100 gm. Latex	—	—	—	—	—	—

Fields 3/5 and 4/5.

Fields V and 3/5 & 4/5 Together	N% T.S.	-	Modulus	+ 0.93
	N% Rubber	-	Modulus	+ 0.75
	D.R.C.	-	Modulus	− 0.94
	N% T.S.	-	D.R.C.	− 0.94

H S P = 0.01

S P = 0.05

N S P > 0.05

Correlation between Latex Properties

NITROGEN—D.R.C.

Nitrogen of latex is expressed as per cent of the total solids content.

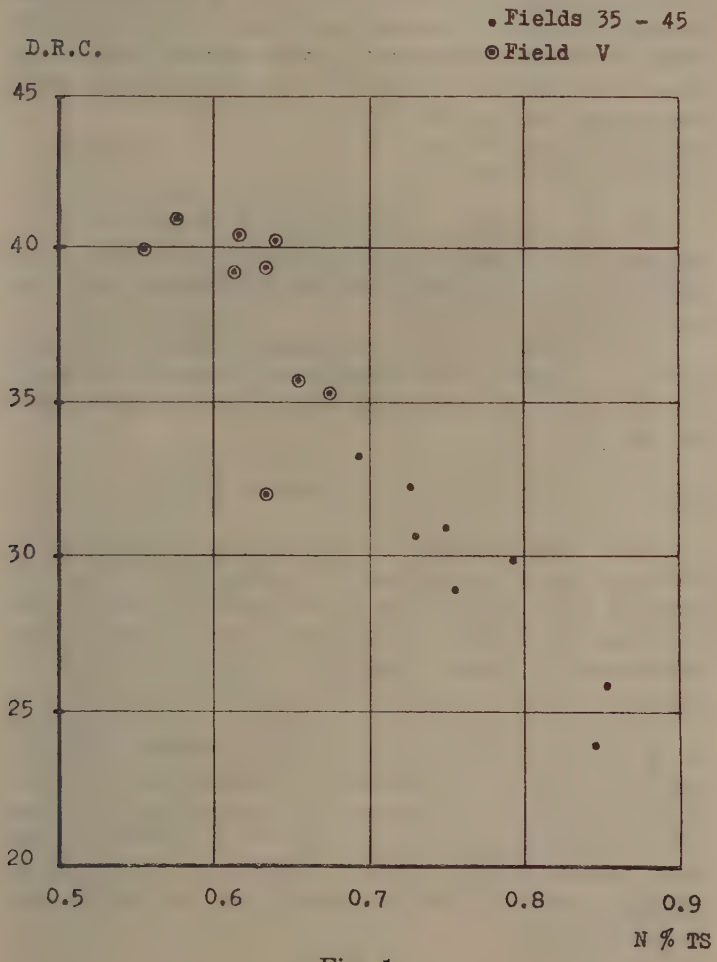

Fig. 1

If we look at the results obtained separately for field V on the one hand, and for fields 3/5 and 4/5 on the other hand, we observe a highly signifianct negative correlation in latex from young trees, and a non-significant correlation in that from the 15 year old trees. But if we take all the results as a single population, we find a

correlation more highly significant than obtained for fields 3/5 and 4/5 alone. The low value for the coefficient found for field V is not surprising as from Figure 1 we see that for 9 points (9 means from 9 clones), 6 have a D.R.C. ranging from 39.6 to 40.8% and a nitrogen content from 0.61 to 0.57%. It may therefore be that errors due to the methods of determining D.R.C. and nitrogen can mask the correlation. At the same time, we have observed bigger differences between the properties of latex from young trees tapped during the first year than for older ones under tapping for 10 years.

Nitrogen—Total Solids

We also obtain a highly significant correlation when we use all the results available from the various fields, although we do not find significant correlation if the fields are considered separately.

With regard to the relationship between nitrogen content and total solids, attention is drawn to the curve recently given by Mc Colm (*Rubber Age*, New York, 1950, 34, 431-436).

Copper Content—D.R.C., Total Solids, Nitrogen

The copper content of latex was determined as milligrammes of copper per 100 grammes of latex, the analyses being carried out on weighed samples of latex for practical reasons. We have recently re-calculated, expressing the copper in per cent of total solids and find figures very close to the previous results.

The systematic analyses of copper were commenced at a later date than those of nitrogen. We have only results for the field V latices and therefore they are given with considerable reservation. We observe negative correlations between copper and both D.R.C. and total solids, the former being highly significant. A significant positive correlation between copper and nitrogen content is also found.

What we have observed above about the nitrogen—D.R.C. correlation for the latex of field V is also here applicable and a significant correlation is particularly remarkable, considering the very small copper values, ranging only from 0.19 to 0.34%. Since our results are very limited, they do not permit further discussion. It will be interesting at a later date to compare our results with the important study already made by G. V. van der Bie about nitrogen and copper in rubber (Thesis Amsterdam 1948).

Correlation between Latex Properties and Rubber Modulus

Total Solids Nitrogen—Modulus

The correlation is positive and highly significant for each field, it is most highly significant if we consider all the results together.

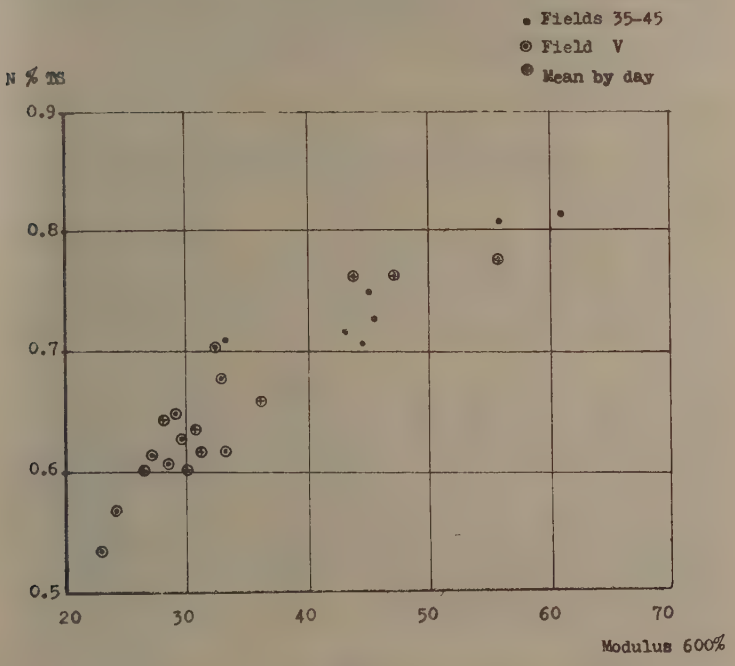

Fig. 2

The correlations have been calculated using the means of each clone for all tapping days. This eliminates to some extent the day to day variations due to climate or

seasonal conditions. We have also included in Figure 2 a number of correlation points corresponding to the means of all clones for different days, thus lessening the genetic factor and emphasising the climate effect.

It is seen that these points are in agreement with the others and do not modify the correlation, however owing to the short period over which the observations are made, it is not possible to draw definite conclusions. Wide variation in the same field between nitrogen content of the latex and the rubber modulus from one tapping day to another is evident, particularly for the young clearing.

It is interesting to compare the latex nitrogen—modulus correlation with the rubber nitrogen—modulus correlation. It has been previously reported after examining more than one hundred rubber samples from different estates that a significant correlation exists. From these more recent results for which nitrogen in latex was analysed as well as on the rubber, we notice contrary to expectation that the correlation is more significant for total solids nitrogen than for rubber nitrogen.

D.R.C.—Modulus

The correlation is negative and highly significant for each field, it is most highly significant when all the results are pooled.

As for the total solids nitrogen—modulus correlation, we have added in Figure 3 several points, each corresponding to the mean of the fields for one tapping day. We notice that these points do not fit so well as in the other case.

In this connection we can observe that the D.R.C. of latex may be affected without involving the latex metabolism. For example, wetting of the panels will cause a decrease of the D.R.C. and in very dry weather a slight increase may occur. We therefore consider that the relationship found between total solids nitrogen and modulus may be of more practical interest than that between D.R.C. and modulus.

Conclusions

The results reported above are corroborated by the work of P. Tixier. In his paper relating to copper

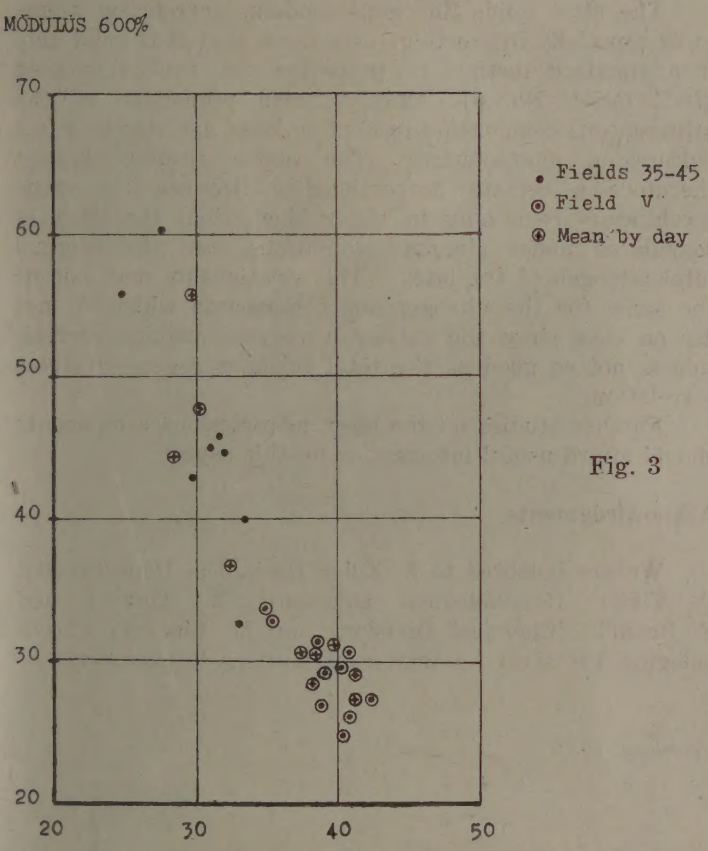

Fig. 3

sulphate injections into the trees, he reports a slight increase in copper content of the latex, a decrease of D.R.C., an increase of the rubber modulus and from the few analyses made an increase in the total solids nitrogen content.

It would therefore appear that total solids copper and nitrogen contents, D.R.C. and rubber modulus are connected, but it is not yet possible to state whether they are directly connected, or the limits in which this may be valid.

The correlations between nitrogen, copper and D.R.C. concern latex physiology and possibly even the rubber metabolism, but to speculate on this subject is most premature.

The total solids nitrogen—modulus correlation seems to us especially interesting. We know that it is valid only if a standard method of preparing the rubber is used (*Bull. Caout.* No. 6). Only a small proportion of the nitrogeneous components present in latex are able to affect vulcanising characteristic. The above results suggest therefore a certain proportionality between the basic accelerators remaining in the rubber when the latex is coagulated under standard conditions, and the original total nitrogen of the latex. This relationship may not be the same for the nitrogeneous components which do not act on cure since the rubber nitrogen—modulus correlation is not so good as the total solids nitrogen—modulus correlation.

Further studies on the latex nitrogeneous components should afford useful information on this aspect.

Acknowledgments

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